EVALUATION OF TOXICITY AND ANTIULCER ACTIVITY OF ETHANOLIC EXTRACT OF *ECHINOPSECHINATUS*ROXB.

A Dissertation submitted to

THE TAMIL NADU Dr.M.G.R. MEDICAL UNIVERSITY
CHENNAI - 600 032

In partial fulfillment of the requirements for the award of the Degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

Submitted By
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Under the guidance of

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CERTIFICATE

This is to certify that **Mr. SYEDIKRAMSHARIF S R** with University Registration no**. 261626002** carried out the dissertation work entitled "**Evaluation of toxicity and antiulcer activity of ethanolic extract of aerial parts of *Echinops echinatus* Roxb"** for the award of degree in Master of Pharmacy by The Tamil Nadu Dr. M.G.R Medical University**.** The dissertation is a bonafide work done by the above said student under the supervision of Dr. C. Ronald Darwin., Professor & Head Department of Pharmacology. The work embodied in this thesis is original and has not been submitted in part or in full for any degree of this or any other university.

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DECLARATION

I **SYEDIKRAMSHARIF S R** hereby declare that the dissertation work entitled "**Evaluation of toxicity and antiulcer activity of ethanolic extract of aerial parts of *Echinops echinatus* Roxb"** for the award of degree in Master of Pharmacy by The Tamil Nadu Dr. M.G.R Medical University**.** The dissertation is a bonafide work done by me under the supervision of **Dr. C. Ronald Darwin. M.Pharm., Ph.D., Professor & Head Dep. of Pharmacology.** The work embodied in this thesis is original and has not been submitted in part or in full for any degree of this or any other university.

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Internal Examiner **External Examiner**

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SYEDIKRAMSHARIF S R

TABLE OF CONTENTS

I. INTRODUCTION

PEPTIC ULCER:

Peptic ulcer is one of the most common gastrointestinal disorders, causes high rate of morbidity particularly in the population of non-industrialized countries[1].Peptic ulcer is caused due to an imbalance between the aggressive (pepsin, acid, and Helicobacter pylori) and the defensive (gastric mucus and bicarbonate secretion, prostaglandins, innate resistance of the mucosal cells) factors[2]. New etiological factors, such as involvement of free radicals and *H. Pylori* bacteria.

Basically, word “peptic” is derived from Greek term “peptikos” whose meaning is related to digestion[3]. Normally, the lining of the stomach and small intestine is protected against the irritating acids produced in your stomach. If this protective lining breaks off in the continuity it results in inflammation (gastritis) or an ulcer[4].

Peptic ulcer can develop into more serious disorders. As a result of complications, peptic ulcer may lead to perforate or bleeding ulcer[5].Perforate ulcer involves severe tearing of the mucosal membrane, causing acute pain and sourness at the abdominal level. Bleeding ulcer involves internal haemorrhages and it needs immediate medical intervention. The cause of ulceration in patients is mainly due to hypersecretion of gastric juice or due to hypersecretion of pepsin[6]. Less common form of peptic ulcers are zollinger–Ellison syndrome ulcers, drug associated ulcers and stress ulcers, *H. Pylori*.

In Ayurveda, peptic ulcer mostly refers to *Amlapitta* or Parinamasula*. Amlapitta* is a disease of the gastrointestinal tract, especially of the stomach. It has not been described as an independent disease in major Ayurvedic texts, but has been mentioned in short in *Kashyapasamhita. Amlapitta* literally means, pitta leading to sour taste[7].Apart from the stressplaced on food habits and personal hygiene, some herbal drugs have also been mentioned in the treatment of this disease.

Indian medical plants and their derivatives have been shown invaluable source of therapeutic agents for the treatment of various disorders including peptic ulcer disease. The indigenous drug possessing lesser side effects is the major

thrust area of the present day research, aiming for a better and safer approach for the management of peptic ulcer diseases.

REGULATION OF ACID SECRETION BY PARIETAL CELLS[8]:

The regulation of acid secretion by parietal cells is especially important in the pathogenesis of peptic ulcer. The three major pathways for the regulation of parietal acid secretions include.

1) Neural stimulation in the vagus nerve.
2) Endocrine stimulation via gastrin released from antral G cells and.
3) Paracrine stimulation by local release of histamine from enterocromaffin like(ECL) cells.

The secretion of the parietal cells is an isotonic solution of HCL with pH less than 1, the concentration of hydrogen ions being more than a million times higher than that of the plasma. The Cl^- is actively transported into canacliculi in the cells that communicate with the lumen of the gastric glands and thus with the stomach itself. This Cl^- secretion is accompanied by K^+, which is then exchanged for H^+ from within the cell by a K^+/H^+ ATPase (fig: 1) . An increase in the permeability of the apical membrane to K+ &Cl^- accompanies activation of proton pump, the final step of HCL production is operated by the gastric proton pump[9].

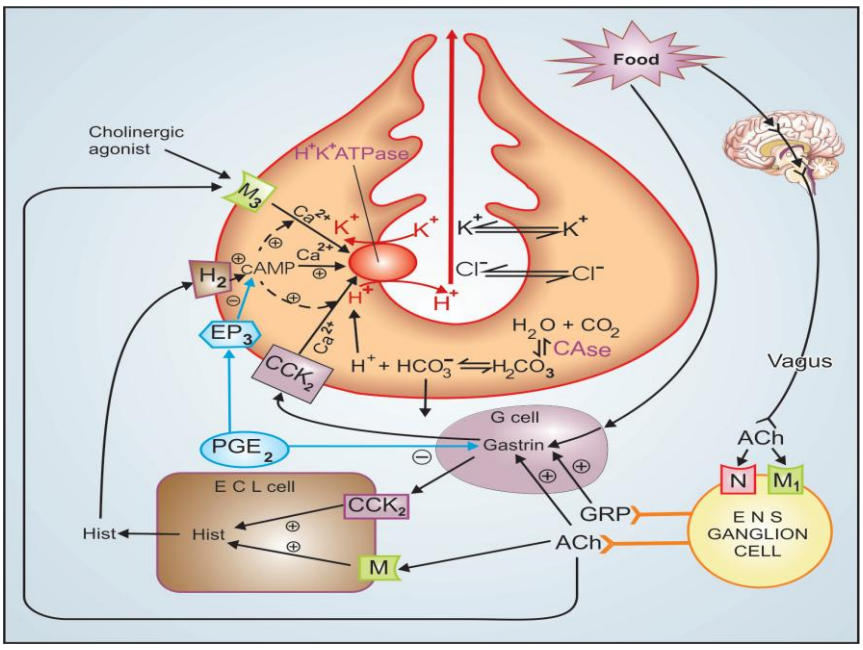


Figure: 1 A schematic illustration of the secretion of HCL by the gastric parietal cells. Proton pump (P), Symport carrier (C), Antiport (A)

Carbonic anhydrase catalyses the combination of carbon dioxide and water to give carbonic acid, which dissociates into H^+ and bicarbonate ions. The latter exchange across the basal membrane of the parietal cell for Cl^-. The principle stimuli acting on the parietal cells are:

- Gastrin (a stimulatory hormone)
- Acetylcholine (a stimulatory neurotransmitter)
- Histamine (a stimulatory local hormone)
- Prostaglandins E_2 and I_2 (local hormone that inhibit acid secretion)

Chemical Mediators:

Gastrin:

Gastrin is a peptide hormone synthesised in endocrine cells of the mucosa of the gastric antrum and duodenum, and secreted into the portal blood. Itsmain action is to stimulate the secretion of acid by the parietal cells.

Gastrin also indirectly increases pepsinogen secretion, stimulates blood flow and increases gastric motility. Release of this hormone is normally controlled by neurotransmitters and blood brain barriers.

Acetylcholine:

Acetylcholine is released from neurons and stimulates specific muscarinic receptors on the surface of the histamine-containing cells.

Histamine:

Within the stomach, mast cells (or histamine containing cells similar to mast cells) lying close to the parietal cell release a steady basal release of histamine, which is further increased by the gastrin and acetylcholine. The hormone acts on the parietal cell H_2 receptors, which are responsive to histamine concentrations that are the threshold require for vascular H_2 receptors activation. The histamine inturn activates parietal cells H2 receptors that are linked to the stimulation of adenylcyclase causing activation of the cAMP pathway[10].

The exact mechanism of action of the three secretagogues on the parietal cell is not clear. A general scheme is given in Fig: 2, which summarises the two main theories: the single cell or permission hypothesis and two cell or transmission hypothesis. According to the former concept, the parietal cell itself has H2

receptors for histamine and muscarinic M2 receptors for acetylcholine, as well as receptors for gastrin itself. Acid secretion as follows after the synergistic of H2 (which increases cAmp) receptors and M2 and gastrin receptors (which increases cytosolic Ca^{2+})

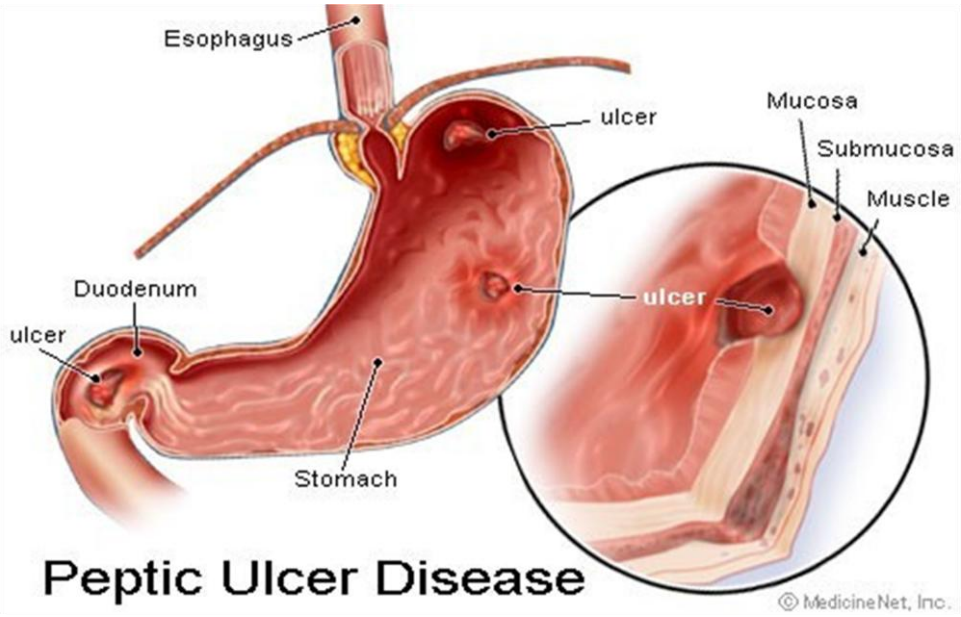


Figure: 2

CAUSES:

Helicobacter pylori:

H. pylori bacteria lives and multiply within the mucous layer that covers and protect tissues that line the stomach and small intestine. Often, *H. pylori* causes no problems. But sometimes it can damage the mucous layer and inflame the lining of your stomach or duodenum, leads to formation of ulcer. *H. pylori*may be transmitted from person to person by close contact, such as kissing. People may also contact *H. pylori* through Water and food [11].

NSAIDS:

NSAIDS are non-steroidal anti-inflammatory drugs. The most commonly known NSAIDS are ibuprofen, aspirin, and naproxen sodium. Others are prescription NSAIDs used to treat several arthritic conditions. NSAIDS can make the stomach's defence mechanisms to fail in a couple of different ways:

- They can make the stomach vulnerable to the harmful effects of acid and pepsin by interfering with the stomach's ability to produce mucus and bicarbonate.
- NSAIDS can affect cell repair and blood flow to the stomach.

The gastric mucosa protects itself from gastric acid with a layer of mucus, the secretions are stimulated by certain prostaglandins. NSAIDs block the function of COX- 1(cyclooxygenase -1), which is essential for the production of these prostaglandins. COX-2 selective anti-inflammatories preferentially inhibit COX-2, which is less essential in the gastric mucosa, and roughly have the risk of NSAID - related gastric ulceration[12].

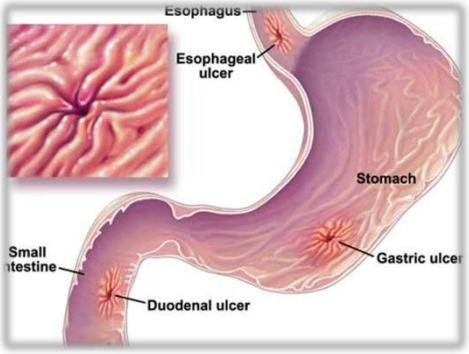
ACID AND PEPSIN:

Acid and pepsin are powerful digestive fluids and are believed to contribute to the formation of ulcers. In ideal situations, the stomach can protect itself from these fluids in several ways. These are:

- The stomach produces lubricant-like mucus that coats the stomach and shields stomach tissues.
- The stomach can produce chemical called bicarbonate that neutralizes digestive fluids and breaks them down into less harmful substances.
- Blood circulation in the lining of the stomach, as well as cell renewal and repair, help protect the stomach[13].

SMOKING:

Studies show that cigarette smoking can increase a person's chance of getting an ulcer.Smoking can also slow down the healing of existing ulcers and contributes to ulcer reoccurrence.Smoking by itself may not be much of a risk factor unless the presence of *H.pylori* infection[14].



ALCOHOL:

Alcohol contains chemicals which are similar to the gastric acid that our pancreas produce in order to emulsify the food we eat. Alcohol can also contribute or trigger to cause gastric wounds in the lining of your stomach causing peptic ulcers[15].

STRESS:

Emotional stress is no longer thought to be a cause of ulcers, people who are experiencing emotional stress often report increased pain of existing ulcers. However Physical stress, is different. It can increase the risk of developing ulcers, especially in the stomach. Examples of physical stress that can lead to ulcers are that suffered by people with injuries such as severe burns, and people undergoing major surgery[16].

DIET:

No convincing evidence shows that certain diets can cause ulcers or that certain diets can heal ulcers and keeps them healed. A diet may help to relieve the pain or indigestion of an existing ulcer, but it will not prevent an ulcer from forming. Many foods that initially neutralize acid in the stomach also may stimulate additional acid secretion. In fact, research has showed that milk, which was a mainstay in the diets of ulcer patients, actually can be a potent stimulant of gastric acid secretion. Absence of protective factors like fibres in the diet causes peptic ulcer[17]. The accumulation of Histamine and Tyramine in food by microbial action and their concentration in various food, wine, beer, and fermented products also cause hyperacidity and ulceration.

BILE REFLUX:

The increased concentration of bile salts in stomach interferes with the integrity of gastric mucosa thus, predisposing to the development ofulceration[18].

POOR BLOOD SUPPLY:

Resistance of mucosa to digestion is reduced as a result of impaired blood supply. Such impairment could occur as a result of venous or arterial thrombosis or because of “shunting” of blood within the mucosa[19].

HEREDITY:

A strong family history is frequently found in patients who develop ulcer in childhood or adolescence. It was difficult to be certain of the diagnosis of an ulcer before radiology was developed and because the prevalence of the condition makes the chance association of several cases in a family reasonably probable[20].

SEX INCIDENCE:

Duodenal ulcers occur 5-10 times more often in men than in women and the perforation is 20 times more often in men. Although, these effects may be due to differences in life pattern of men and women, there are grounds for supposing that female sex hormones are in some way protective against peptic ulcer[21].

CONSTITUTIONAL FACTORS:

There is sexual difference in occurrence of peptic ulcer. Peptic ulcer occurs at all ages in men. The incidence is very high (80%) in men compared with women. Ulcers are rare in women during there productivity age and particularly during pregnancy. Ulcers may occur in women after menopause[22].

OCCUPATION:

Duodenal ulcers are found to be more common among the physicians and business executives than among other professionals. The incidence is low in agricultural labourers. Other factors responsible for ulceration are chronic lung diseases, chronic liver diseases and hyper parathyroidism. Number of parietal cells present in stomach and hydrochloric acid secretion plays important statistically significant role in ulcer formation. Latest information suggests the role of bacterial organism, Helicobacter pylori which colonize the gastric mucosa, particularly the antral region in the development of chronic ulceration[23].

GASTRITIS:

Gastritis precedes the development of the ulcer. Inflammation causes the development of ulcer by altering the resistance of mucosa to digestion[24].

CLASSIFICATION OF ULCERS[2]:

A peptic ulcer may arise at various locations

Stomach (called gastric ulcer)

Duodenum (called duodenal ulcer)

Esophagus (called esophageal ulcer)

A Meckel's diverticulum.

Most Common Types of Ulcers:

Peptic Ulcer:

Any ulcer that is exposed to pepsin is referred to as peptic ulcers. Peptic ulcers are found in the lining of your stomach or duodenum. Pepsin is normally present along with hydrochloric acid in the stomach lining.

Gastric Ulcer:

When a peptic ulcer is in your stomach, it is called a gastric ulcer. The symptoms of gastric ulcers are more specific than peptic ulcer symptoms.

Duodenal Ulcer:

When a peptic ulcer caused in duodenum, it is called a duodenal ulcer. This type of peptic ulcer develops in the first part of the small intestine. Some of the symptoms of a duodenal ulcer are interestingly quite opposite to those of gastric ulcers. Duodenal ulcers are the most common ulcers found in the Western world.

Lesser Known types of Ulcers:

Esophageal Ulcer:

This type of ulcer occurs in the lower end of your esophagus. Esophageal ulcersare often associated with a bad case of acid reflux, as it is commonly called (short for Gastro Esophageal Reflux Disease).

Bleeding Ulcer :

Internal bleeding is caused by a peptic ulcer which has been left untreated. When this happens, it is referred to as a bleeding ulcer this is the most dangerous type of ulcer. Vomiting of blood indicates that a gastric ulcer started bleeding. This may also cause repeated episodes of nausea.

Refractory Ulcer:

Refractory ulcers are simply peptic ulcers that have not healed after at least 3 months of treatment.

Stress Ulcer:

Stress ulcers are a group of lesions (or lacerations) found in the esophagus, stomach or duodenum. These are normally found only in critically ill or severely stressed patients.

SIGNS AND SYMPTOMS[25]:

Pain is the most common symptom of peptic ulcer. Burning pain is the most common. The pain caused by the ulcer is aggravated by stomach acid coming in contact with the ulcerated area.

Abdominal pain, classically epigastric strongly correlated to mealtimes. In case of duodenal ulcers the pain appears about three hours after taking a meal.(duodenal ulcers are classically relieved by food, while gastric ulcers are exacerbated by it)

Bloating and abdominal fullness;

Water brash (rush of saliva after an episode of regurgitation to dilute the acid in oesophagus - although this is more associated with gastroesophageal reflux disease);

Nausea, and copious vomiting;

Hematemesis (vomiting of blood); this can occur due to bleeding directly from a gastric ulcer, or from damage to the esophagus from severe/continuing vomiting;

Loss of appetite and weight loss;

Melena (tarry, foul-smelling faeces due to oxidized iron from haemoglobin);

Rarely, an ulcer can lead to a gastric or duodenal perforation, which leads to acute peritonitis. This is extremely painful and requires immediate surgery.

The symptoms of peptic ulcers may vary with the location of the ulcer and the patient's age. Furthermore, typical ulcers tend to heal and recur and as a result the pain may occur for few days and weeks and then wane or disappear. Usually, children and the elderly do not develop any symptoms unless complications have arisen.

The timing of the symptoms in relation to the meal may differentiate between gastric and duodenal ulcers.

A gastric ulcer would give epigastric pain during the meal, as gastric acid production is increased as food enters the stomach.

Symptoms of duodenal ulcers would initially be relieved by a meal, as the pyloric sphincter closes to concentrate the stomach contents; therefore acid is not reaching the duodenum.

Duodenal ulcer pain would manifest mostly 2–3 hours after the meal, when the stomach begins to release digested food and acid into the duodenum.

COMPLICATIONS[26]:

1. BLEEDING:

As an ulcer erodes into the muscular portion of the gastric or duodenal wall, it can erode into blood vessels and cause bleeding into the digestive tract. If the damaged blood vessels are small, the blood may seep out slowly, and over a long period of time, the patient can gradually become anemic. On the other hand, if the damaged blood vessel is large, bleeding into the intestinal tract is more rapid and can be very dangerous. The patient may feel faint, vomit blood, or collapse suddenly. With some bleeding ulcers, the stool may become a tarry black colour due to the digested blood it contains. Without prompt medical attention, often including blood transfusions and surgery, the patient may bleed to death[27].

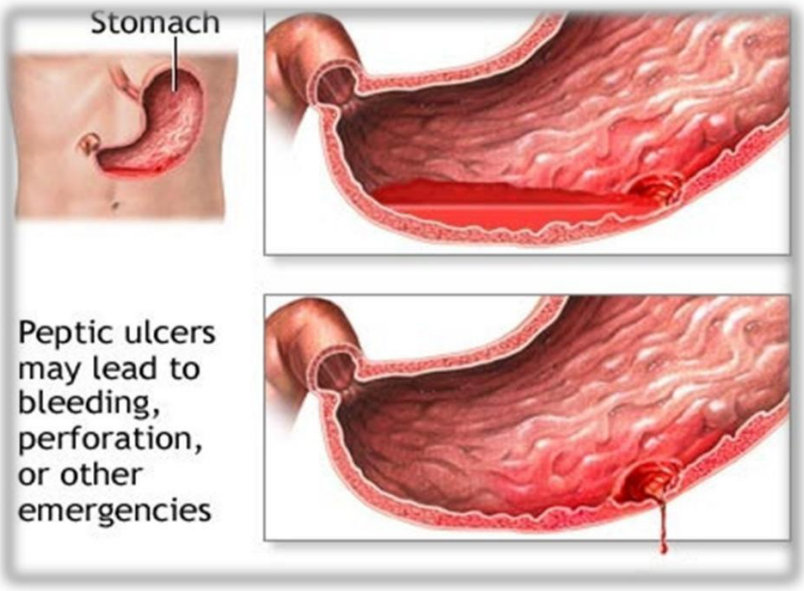


Figure: 4

2. **PERFORATION:**

Sometimes an ulcer will erode all the way through the wall of the stomach or duodenum. If this happens, partially digested food and bacteria from the digestive tract can spill into the sterile abdominal cavity and cause peritonitis, an inflammation of the abdominal cavity and wall. A perforated ulcer, which can cause sudden, severe pain, usually requires hospitalization and corrective surgery[28].

3. NARROWING AND OBSTRUCTION:

Ulcers that occur in the duodenum or in the narrow section where the stomach connects to the duodenum can cause spasms of the adjacent muscles and swelling of surrounding tissue. This swelling can cause the intestinal opening to become narrowed or closed off completely. Such an obstruction can prevent food from leaving the stomach and entering the intestinal tract. A patient may vomit the contents of the stomach and, if the condition continues, lose weight and develop other problems. Again, surgery may be necessary to correct this problem.

DIAGNOSIS[29, 30]:

Esophagogastroduodenoscopy (EGD) a form of endoscopy, also known as a gastro copy in which a fiber optic tube with an attached camera is threaded down the throat and esophagus into stomach and duodenum. With this sensitive method, the upper digestive tract is viewed, ulcer can be identified.

- Upper gastro intestinal (upper GI) X-ray which involves swallowing a white metallic liquid (containing barium) that coats the digestive tract and makes an ulcer more visible.
- Direct culture from an EGD biopsy specimen. The specimen is examined under a microscope to rule out cancer. A biopsy can also identify the presence of *H.pylori* in stomach lining
- Direct detection of urease activity in biopsy specimen-Rapid urease test (CLO test).

H. pylori infection is most commonly diagnosed with following tests:

- **Blood test (Serological tests):** IgG antibodies against *H.pylori* can be detected inthe serum using laboratory based and near patient testing serology kits.

A disadvantage of this test is that it sometimes can't differentiate between part exposure and current infection. After *H. pylori* bacteria has been eradicated, still a positive result may be obtained for many months.

- **Breath test:** Isotopically labelled carbon dioxide can be detected in the breath of*H. pylori* positive individuals following ingestion of urea labelled with 13_C or 14_C.

TREATMENT OF PEPTIC ULCER[2]:

1. Reduction of gastric acid secretion :
   a) H_2-antihistamines **:**Cimetidine, Ranitidine, Famotidine, Roxatidine, Loxatidine
   b) Proton pump inhibitors **:**Omeprazole, Lansoprazole, Pantoprazole, Rabeprazole, Esomeprazole
   c) Anticholinergics**:** Pirenzepine, Propantheline, Oxyphenonium
   d) Prostaglandin analogues**:**Misoprostol, Enprostil, Rioprostil
2. Neutralization of gastric acid (antacids) :
   a) Systemic **:** Sodium bicarbonate, Sodium citrate
   b) Non systemic **:** Magnesium hydroxide, Magnesium trisilicate, Aluminium hydroxide gel, Magaldrate, Calcium carbonate
3. Ulcer protective **:** Sucralfate, Colloidal bismuth subcitrate (CBS)
4. Ulcer healing drugs **:** Carbenozolone Sodium
5. Anti H-Pylori drugs**:** Amoxycillin, Clarithromycin, Metronidazole, Tinidazole, Tetracycline.

MECHANISM OF ACTION:

- **H_2 receptor antagonists**: These drugs act by competitively inhibit histamine actionsat all H_2 receptors, but their main clinical use are as inhibitors of gastric acid secretion. They can inhibit histamine, gastrin, and acetylcholine stimulated acid secretion; pepsin secretion also falls with the reduction in volume of gastric juice.
- **Proton pump inhibitors:** The parietal cells secrete $H^+$with the help of a membranelocated enzyme H^+ K^+ ATPase called as proton pump or an acid pump. These drugs inhibit the proton pump and abolish HCl secretion. The

drug is a week base andaccumulates in the acid environment of the canaliculi of the stimulated parietal cell were it is activated.

- **Anti cholinergic drugs:** There drugs acts as selective antagonist at M_1muscarinicreceptors. These drugs reduce the volume of gastric juice without raising its pH unless there is food in stomach to dilute the secreted acid. Stimulated gastric secretion is less completely inhibited.

- **Prostaglandin analogues:** PGE and PGI reduce the secretion of gastric acid and atlower concentrations may promote cytoprotection. Inhibit gastrin production, increase mucosal blood flow and probably have an ill-defined "cytoprotective" action. However, the most important appears to be their ability to reinforce the mucus layer covering gastric and duodenal mucosa which is buffered by HCQ] secreted into this layer by the underlying epithelial cells.

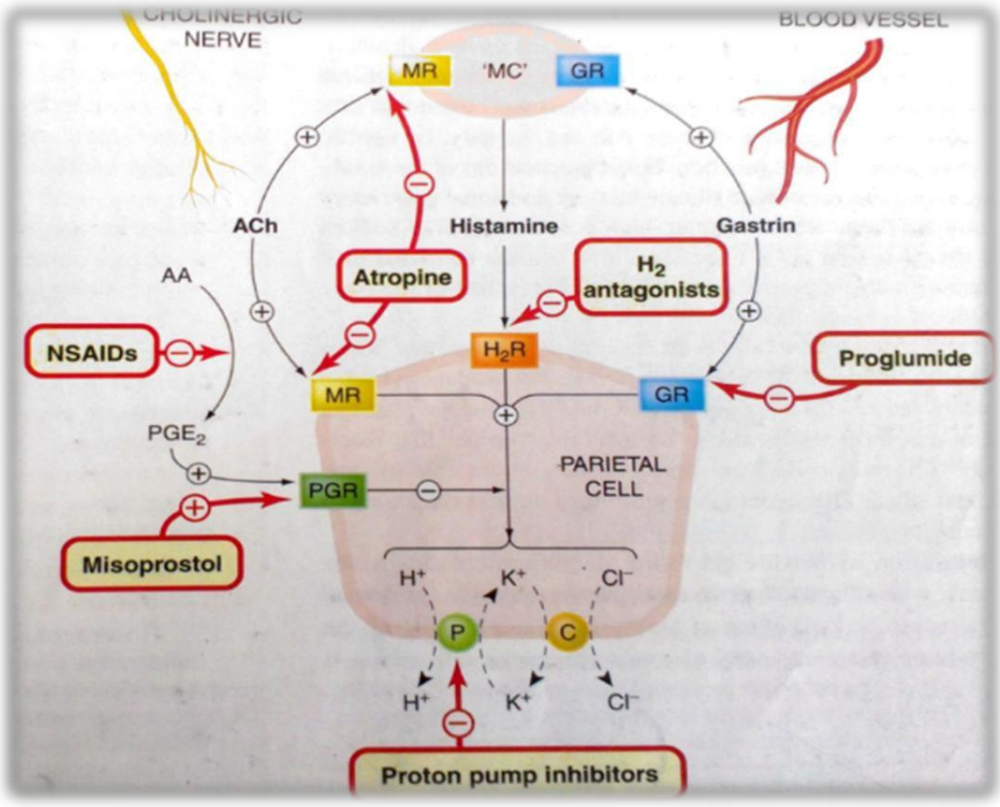


Figure: 5

- **Systemic antacids:** They are absorbed into the systemic circulation and causealkalosis. It is water soluble, acts instaneously, but the duration of action

is short. It is a potent neutralizer, pH may rise above 7 However, it has several demerits:

- Absorbed systemically: large doses will induce alkalosis
- Acid rebound occurs, but is usually short lasting
- Produces $C0_2$ in stomach distention, discomfort, belching, risk of ulcer perforation.
- Increases Na^+ load: may worsen edema and contraindicated in cardiac diseases
- **Non-systemic antacids:** In stomach, the cation of the antacid forms a chlorideduring neutralization of the acid. In the alkaline pH of small intestine, the chloride salt reacts with the bicarbonate from the intestinal juices to regenerate original salt. Hence systemic alkalosis is avoided
- **Ulcer protective's:** They precipitate surface proteins at an ulcer base and acts as anacid resistant physical barrier preventing acid, pepsin and bile from coming in contact with the ulcers.
- **Ulcer healing drugs:** They cause greater production of gastric mucus by increasingendogenous PG which has a protective and coating action.

SCREENING METHODOLOGIES FOR ANTIULCER ACTIVITY:

In Vivo Methods:

1. Pylorus Ligation In Rats (Shay model)
2. Stress Ulcer Through Immobilization Stress
3. Stress Ulcers By Cold Water Immersion
4. Indomethacin Induced Ulcers In Rats
5. Ethanol Induced Mucosal Damage In Rats (Cytoprotective Activity)
6. Subacute Gastric Ulcer In Rats
7. Gastric Ischemia-Reperfusion Injury In Rats

1. Pylorus ligation in rats (SHAY) :

Male or Female Wistar strain albino rats weighing 150-170g are starved for 48 hrs, having access to drinking water and libitum. During this time they are

housed single in cages with raised bottoms of wide wire mesh in order to avoid cannibalism and coprophagy. Sixanimals are used per dose and as controls. Under ether anesthesia a midline abdominal incision is made. The pylorus is ligated, care being exercised that neither damage to the blood supply nor traction on the pylorus occurs. Grasping the stomach with instruments is to be meticulously avoided; else ulceration will invariably develop at such points. The abdominal wall is closed by sutures. The test compounds are given either orally by gavage or injected subcutaneously.

The animals are placed for 19hrs in plastic cylinders with an inner diameter of 45 mm being closed on both ends by wire mesh. Afterwards, the animals are sacrificed in CO_2anesthesia. The abdomen is opened and a ligature is placed around the esophagus close to the diaphragm. The stomach is removed and the contents are drained in a centrifuge tube. Along the greater curvature the stomach is opened and pinned on a cork plate. The mucosa is examined with a stereomicroscope. In the rat, the upper two fifths of the stomach form the Lumen with squamous epithelium and posses little protective mechanisms against the corrosive action of gastric juice. Below a limiting ridge, in the glandular portion of the stomach, the protective mechanisms are better in the mucosa of the medium two fifths of the stomach than in the lower part, forming the antrum. Therefore, lesions occur mainly in the lumen and in the antrum. The number of ulcers is noted and the severity recorded with the following scores

O = no ulcers

1 = superficial ulcers

2 = deep ulcers

3 = perforation

The volume of the gastric content is measured. After centrifugation, acidity is determined by titration with 0.1 N NaOH.

2. Stress ulcer through remobilization stress:

Groups of 6 female Wistar rats per dose of test drug and for controls weighing 150-170g are used. Food and water are withdrawn 24hrs before the experiment. After oral or subcutaneous administration of the test compound or the placebo solution the animals are slightly anaesthetized with ether. Both lower and

upper extremities are fixed together and the animals are wrapped in wire gaze. They are horizontally suspended in the dark at 20□C for 24 hrs. and finally sacrificed in CO_2anesthesia. The stomach is removed, fixed on a cork plate and the number and severity of ulcers in registered with a stereo microscope using the following scores.

0 – no ulcers

1 – Superficial ulcers

2 – Deep ulcers

3– Perforation

3. Stress ulcers by cold water immersion:

Groups of 8–10 Wistar rats weighing 150-200g are used. After oral administration of the test compound the rats are placed vertically in individual restraint cages in water at 22□C for one hour. Then, they are removed, dried and injected intravenously via the tail vein with 30mg/kg Evans blue. Ten min later, they are sacrificed in CO_2anesthesia and their stomachs removed. Normal saline (2% v/v) is then injected into the totally ligated stomachs for storage overnight. The next day, the stomachs are opened along the greatest curvature, washed in warm water and examined under a 3-fold magnifier. The lengths of the longest diameters of the lesions are measured and summated to give a total lesion score (in mm) for each animal, the mean count for each group being calculated.

4. Indomethacin induced ulcers in rats:

Groups of 6 Wistar rats weighing 150-200g are used. The test drugs are administered orally in 0.1% Tween 80 solution 10 min prior to oral indomethacin in a dose of 20 mg/kg (4mg/ml dissolved in 0.1% Tween 80 solution). Six hours later, the rats are sacrificed in CO_2anesthesia and their stomachs removed. Normal – saline (2% v/v) is then injected into the totally ligated stomachs for storage overnight. The next day the stomachs are opened along the greater curvature, then washed in warm water and examined under a 3-fold magnifier. The lengths of the longest diameters of the lesions are measured and summated to give a total lesion score (in mm) for each animal, the mean count for each group being calculated.

5. Ethanol induced mucosal damage in rats:

Male Wistar rats weighing 250-300g are deprived of food 18 hours prior to the experiment but are allowed free access to water. During this time they are kept in restraining cages to prevent coprophagy. The rats are administered either the appropriate vehicle or the cytoprotective drug eg: a prostanoid, intragastrally 30min prior to administration of 1ml absolute ethanol. Untreated animals are included as controls. One hour after administration of ethanol, the animals are euthanized with CO_2 the stomachs are excised, cut along the greater curvature, and gently rinsed under tap water. The stomachs are stretched on a piece of foam core mat, mucosal site up. The subjective scores of the treated tissues are recorded, the graded response is reflected the least (0) to most (3) damage. A circular full thickness area, about 13mm in diameter, is cut with a cork borer from each lobe of the fundus just below the ridge dividing the glandular from the non-glandular portion of the stomach. A Plexiglas template (19 x 14 x 0.3cm) burnished on one side with emery cloth and with four rows with six holes 13mm in diameter is placed on a sheet of clear glass, burnished side up, and bound to the glass with photographic tape along the periphery. The excised pairs of tissue from each stomach are placed into the holes of the template.

Pairs of tissue from each stomach are examined to minimize sampling errors. The template is positioned on a rectangular central open area of Aristo model T-16 cold cathodes transilluminator (38 x38 cm) containing a W-45 blue white lamp. A camera is mounted on a copy stand directly above the template. Photographs are taken; the film processed in a standard manner and a contact sheet is made from the negatives. A light transmission densitometer (eg: MacBeth model TD.501) is used to evaluate the negatives. The optical density of the test tissues is determined by placing each area of the negative in sequence over the aperture through which the light is transmitted. The optical density is displayed on a digital read out and recorded. Hemorrhagic or damaged areas appease bright on the negative whereas undamaged tissue appears dark. Hence, lower optical density values are indicative of damage while higher optical densities are associated with little or as in the case of control, no damage.

6. Subacute gastric ulcer in rats:

Female Wistar rats weighing 120-150g are fasted for 24 hrs. having access to water at libitum in cages with wire sieves at the bottom. The rats are anaesthetized with either and a polyethylene catheter including a fine steel wire with a needle tip (1.2mm diameter) at the lower end is orally inserted into the stomach. After the cannula reaches the gastric wall, the upper end of the steel wire is pressed in a definitive manner, so as to puncture the gastric wall. Each rat is kept in the same position during the intervention in order to locally the puncture at nearly the same region of the glandular part of the stomach. The test substances are administered orally, 30mm or 24 hrs after puncture. Free access to food and water is provided from 2 hours upto the end of the experiment. Each group consists of 8-15 rats.

The animals are sacrificed by overdose of either at definitive time intervals after puncture. The stomach is dissected and opened along the lesser curvature, extensively rinsed in tap water and fixed to the end of a polyethylene tube of 10mm diameter (plastic tip of anautomatic pipette) in or position with the punched ulcer in the centre. The end of the tube with the gastric wall is suspended in a beaker containing physiological saline and the pressure in the tube is gradually increased with a valued rubber ball connected to other end of the tube. The third part of the system is a tonometer calibrated upto 1 bar. The value of tension at which bubbles appear at the ulcerous gastric wall is noted. This value is termed as tensile strength and can be expressed in mmHg.

7. Cysteamine - induced duodenal ulcers in rats:

Male Sprague-Dawley rats with an initial weight of 200g are used. Cysteamine HCl is administered three times on day 1 in a dose of 280mg/kg orally. Protective drugs such as H_2-antagonists are given 30 min prior cysteamine treatment. The rats are sacrificed on the third day. For histological evaluation, the stomach and duodenum are fixed in 10% aqueous buffered formaldehyde and paraffin embedded sections are stained with hematoxylin and eosin. Duodenal ulcers develop in the anterior (antimesentesic) and posterior wall of the proximal duodenum, about 2-4 mm from the pylorus. The more sever ulcers, located on the anterior wall, frequently perforate, resulting in focal or generalized peritonitis or penetrate into the liver. The opposite ulcer invariably penetrates into the pancreas.

In Vitro Method:

Objective:-

To investigate the mechanism of action of a series of potential combination therapies for use against *H. pylori*.

Design:-

The effects of certain antiulcer agents on the antimicrobial activity of antibioticseffective against *H. pylori*were determined In-vitro.

Procedure:-

*H.pylori*was cultured on Skirrow's agar. Amoxycillin Clarithromycin, Erythromycin & Tetracycline were used.

The Antiulcer agents studied by measuring the urease activity. Urease activity was measured by the urease- indophenol method. The minimum inhibitory concentration was determined by a plating method, with H.pylori streaked on plates containing various concentrations of the antibiotics plus sublethal doses of the antiulcer agents, which may be evaluated for the Antiulcer activity.

OXIDATIVE STRESS

A free radical is defined as a molecule or molecular fragments containing one or more unpaired electrons in its outermost atomic or molecular orbital and capable of independent existence[31]. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are describes free radicals and other non-radical reactive derivatives. The reactivity of radicals is generally stronger than non-radical species though radicals are less stable[32].

Free radicals are highly reactive due to the presence of unpaired electrons. Free radicals those are formed during normal metabolism are involved in both human health diseases.

Free radicals are involved in

- Enzyme catalyzed reactions
- Signal transduction and gene expression
- Activation of nuclear transcription factors
- Electron transport in mitochondria

- Oxidative damage to cells , molecules, and tissues
- Anti-microbial action of neutrophils and macrophages
- Aging and disease.

Normal metabolism is dependent upon oxygen, a free radical. Through evaluation, oxygen was chosen as the terminal electron acceptor for respiration. The two unpaired electrons of oxygen spin in the same direction; thus, oxygen is a bi radical, but is not a very dangerous free radical. Other oxygen derived free radical species, such as super oxide or hydroxyl radicals, formed during metabolism or by ionizing radiation are strong oxidants and are therefore more dangerous[33].

In general pro oxidants are referred to as reactive oxygen species. Oxygen derived pro oxidants, which can cause damage to biological target such as

- Lipids
- DNA
- Proteins
- Otherdefending systems of composed of enzymes and reducing equivalents[34]**.**

REACTIVE SPECIES INVOLVED IN OXIDATIVE STRESS AND IMPORTANT ROLE OF FREE RADICALS:

Reactive species mainly classified into three groups:

- Reactive oxygen species
- Reactive nitrogen species
- Reactive chlorine species[35]

Table: 1 Reactive species involved in oxidative stress.

Type	Radicals	Non radicals
ROS	Super oxide, O_2 .-	Hydrogenperoxide,H_2O_2,
	Hydroxyl $OH^{\cdot -}$	Hypochlorus acid, HOCl;
	Peroxyl RO_2.,	Ozone $O_{3;}$
	Alkoxyl RO_2.,	Single oxygen $^1O_2$.
	Hydro Peroxyl HO_2.	
RNS	Nitric oxide (nitric monoxide) $NO^{\cdot}$	Nitrous acid HNO2
	Nitrogen dioxide, NO_2.	Nitrosyl cation NO^+,
		Nitrosyl anion NO^-
		Peroxy nitrite ONOOH
		Alkyl Peroxy nitrite ROONO
RCS	Atomic chlorine ,$Cl^{\cdot}$	Hypochlorus acid HOCl
		Chlorine Cl_2
		Nitronium chloride NO_2Cl

At high concentrations, ROS can be important mediators of damage to cell structures, nucleic acids, lipids and proteins[36]. $O_2^{\bullet -}$ radical is responsible for lipid peroxidation and also have the capability to decrease the activity of other antioxidant defense system enzyme such as catalase (CAT) and glutathione peroxide (GPx), it causes damage to the ribonucleotide which is required for DNA synthesis. $OH^{\bullet}$ radical is most reactive chemical species. It is a potent cytotoxic agent and able to attack and damage almost every molecule found in living tissue. H_2O_2 is not a radical but it produces toxicity to cell by causing DNA damage, membrane disruption and release calcium ions within cell, resulting in calcium dependent proteolytic enzyme to be activated. HOCl isproduced by the enzyme myeloperoxidase in activated neutrophils and initiates the deactivation of antiproteases and activation of latent proteases leading to tissue damage[37].

Super oxide ($O_2^{\cdot -}$) generated in mitochondria, in CVS and others. Fig 6 b) Cells of the immune system (neutrophils, macro phases) also produce the super oxide anion because they contain membrane bound enzyme complex, *respiratory burst* characterized by an increases in oxygen consumption which is accompanied by an increase in glucose utilization and production of NADPH by the pentose phosphate pathway. The NADPH oxidize that reduces oxygen to super oxide

anion once the super oxide anion is formed it can be converted to hydrogen peroxide by the enzyme super oxide dismutase and then it convert to hypochlorus acid by the enzyme myeloperoxidise which can further react with super oxide anion to form the hydroxyl radical. These final products are essential for bacterial killing. Hydroxyl radical very highly reactive, generated during iron over load and such conditions in our body. Hydrogen peroxide formed in our body by large number reactions and yields potent like $^{\cdot}OH$[38].

Peroxyl radical reactive and formed from lipids, proteins, DNA, sugars etc during oxidative damage. Organic hydrogen peroxide reacts with transit metal ions to yield reactive species. Single oxygen highly reactive formed during photosensitization and chemical reactions.

Reactive nitrogen species (RNS) is also a collective term including nitric oxide and nitrogen dioxide radicals and such non radicals as $HNO_{2;}$ ONOOH is often classified as both RNS and ROS. Nitric oxide it is one of neurotransmitter and blood pressure regulator can yield potent oxidants during pathological states. React quickly with almost everything. $RO_2^{\cdot}$ $NO_2^{\cdot}$ And ONOOH have intermediate reactivities.

Nitric oxide is produced in biological tissue by nitric oxide synthase which act as a catalyst to convert L-arginine and oxygen to nitric oxide. Nitric oxide has both pro-oxidant, anti-oxidant properties. For example a nitric oxide radical can both stimulate and inhibit lipid peroxidation. HOCl and NO_2Cl can also be classified as reactive chlorine species are HOCl and $NO_2Cl^{\cdot}$[39].

Fig: 6 a) showing the chemical reaction between nitric oxide and super oxide anion from the highly damaging Peroxy nitrile. Addition of a proton to the Peroxy nitrile leads to the formation of Peroxy nitrous acid. Activated Peroxy nitrous acid can either leads to substrate oxidation and nitration or simply rearrange to nitrate in the absence of adequate substrate[34].

SOURCES OF FREE RADICALS[37, 40]:

The cell is exposed to a large variety of ROS and RNS from both exogenous and endogenous sources.

Endogenous Sources:

- The primary source is from our body during the energy production process within the cells. 98% of the oxygen we breathe is used within our cells in the production of energy. The resultant effect of this energy producing activity is that a very small amount of oxygen that was not used losses electrons creating free radicals.
- Food that we consume may have a destructive potential. An example would be fried foods in high temperature, which causes the bonds in the molecule to become unstable.
- Low blood supply – generally occurs during heart attacks and strokes – forms free radicals.
- During normal metabolism of fat, carbohydrates and proteins, Free radicals may created in the body.
- Stress especially oxidative stress. All chemicals and some endocrine hormones create free radicals.

Exogenous Sources:

- UV radiations, X-rays, gamma rays and microwave radiation.
- Environmental contaminants such as pollution. Cigarette smoke in particular increases free radical load tremendously.
- Unhealthy foods – processed foods, fried foods, barbecued and charbroiled foods.
- Excessive or over-exercising.
- Toxic products found in furniture polish and paints eg., toluene, benzene and formaldehyde
- Industrial effluents, excess chemicals, alcoholic intake, certain drugs, asbestos, certain pesticides and herbicides, some metal ions, fungal toxins and xenobiotics

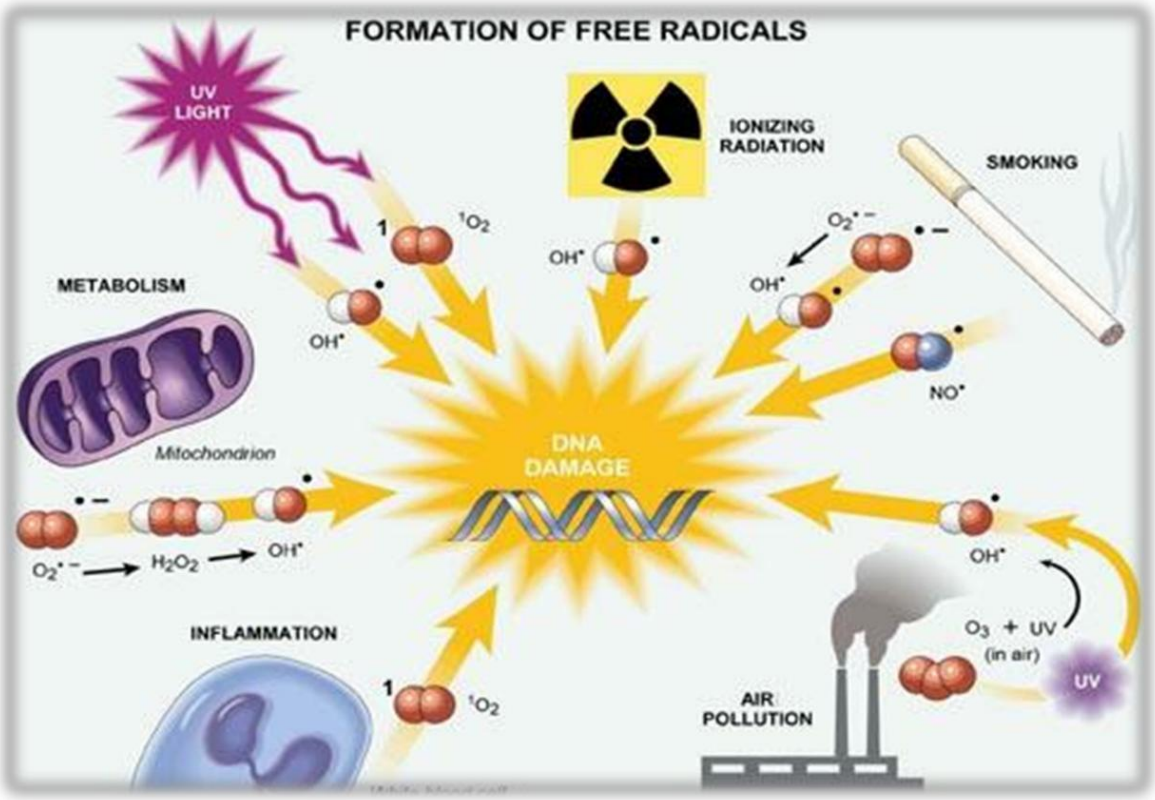


Figure: 7

BIO MARKERS OR SPECIFIC TARGETS OF OXIDATIVE STRESS:

LIPIDS: ROS attack cellular components involving PUFA residue of phospholipids,which extremely sensitive to oxidation. Once formed peroxyl radicals can be re arranged via recyclisation reaction to end peroxide, being MDA the final product. Lipid peroxidation involves LDL as well as HDL oxidation. The process of LP gives many products of toxicological interest like MDA, 4- HNE and various 2 alkenals.

PROTEINS: The side chains of amino acids residues of protein are susceptible tooxidation by the ROS. The protein carbonyl group is generated by ROS through many different mechanisms and its concentration is a good measure of protein oxidation via oxidative stress. The nitric oxide reacts rapidly with $O_2^{\cdot -}$ to form the highly toxic ONOO-that is able o- nitro sate the cystien SH groups of proteins, to nitrate tyrosine and tryptophan residues of proteins and to oxidize methionine residues to methionine sulfoxide. Oxidation of protein is associated with a number of age related diseases and aging.

DNA: Oxidative DNA modifications are frequent in mammalian and have been asimportant contributory factors to the mechanism of carcinogenesis, diabetes and natural aging .The DNA damages are considered as the most serious ROS induced cellular modifications as DNA is not synthesized *denovo* but copied, perpetuating by this way those modifications and hence inducing mutations and genetic instability. The main responsible free radicals such as ·OH and H· react with DNA by addition to bases of hydrogen atoms from the sugar moiety. The C4-C5 double bond of pyrimidine is particularly sensitive to attack by ·OH generating a spectrum of oxidative pyrimidine damage products. Including thymine glycol, uracil glycol 5-OHdU, 5-OHdC, and others. Similarly interact with purine will generate 8-OHdG, 8-OHdA, and other purine oxidative products. Several repair pathway repair DNA damage.8-OHdG has been implicated in diabetes and carcinogenesis and is considered reliable marker for oxidative DNA damage.

CARBOHYDRATES: Free radicals such as·OH react with carbohydrates by randomlyabstracting a hydrogen atom from one of the carbon atoms, producing a carbon centered radical. This leads to chain breaks in important molecules like hyaluronic acid in the synovial fluid surrounding joints, an accumulation and

activation of neutrophils during inflammation produces significant amount of oxy radicals that is also being implicated in rheumatoid arthritis.

OTHER DAMAGES: Oxidative damage to the mitochondrial membrane can also occurresulting in membrane of depolarization and the uncoupling of oxidative phosphorylation with altered cellular respiration. This can ultimately lead to mitochondrial damage, with release of cytochrome c, activation of apoptosis.

REACTIVE OXYGEN SPECIES INDUCED CELLULAR OXIDATIVE DAMAGE:

The continuous efflux of ROS from endogenous exogenous sources results in continuous and accumulative oxidative damage to cellular components and alters many cellular functions. Among the biological targets most vulnerable to oxidative are proteinaceous enzymes, lipidic membrane, and DNA.

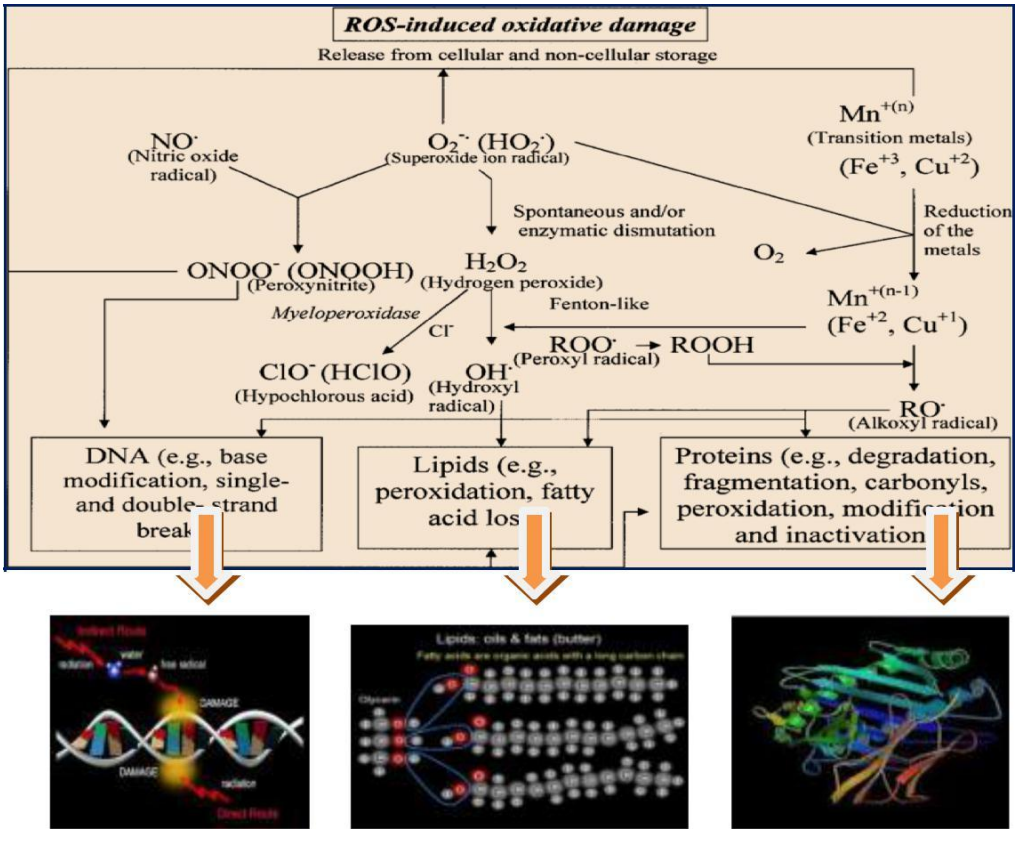


Figure: 8 ROS induced oxidative damage.

Reactive nitrogen species (RNS) like nitric oxide free radical is probably insufficiently reactive to attack DNA. Directly by contrast, di nitrogen trioxide and Peroxy nitrile can lead to deamination of and nitration of DNA. Living organisms

therefore have evolved enzymes that can remove deamination products of Cytosine (Uracil), Adenine (Hypoxanthine) and Guanine (Xanthine) from DNA to decrease the risk of mutagenesis. Excess intra gastric production of RNS (e.g. as result of H.pylori infection chronic

Figure: 9 List of disorders caused by oxidative stress

ANTI OXIDANT DEFENSE SYSTEM:

Antioxidants are any substance that delay or inhibits oxidative damage (oxidation) to a target molecule. The anti oxidant defense which is in action to protect cellular homeostasis against harmful ROS produced during normal cellular metabolism as well as pathological state. Anti oxidant can scavenge free radicals by accepting or donating an electron to eliminate in unpaired condition in most cases the scavenger molecule provides hydrogen radical that combines with free radical. Its generated new radicals thus an enhanced life time compared with the starting one for instance due to a conjugated system. The extended lifetime of this radical by formation of new molecule and thus one scavenger molecule can eliminate second radical.

Many components act against free radicals to neutralize them from both endogenous and exogenous in origin[41].

Endogenous anti oxidants: Super Oxide Dismutase (SOD), Catalase (CAT),Glutathione Peroxidase (GSH-P_X), Glutathione Reductase (GR)

Exogenous anti oxidants: vitamin A and vitamin E & C.

- Super oxide dismutase SOD (scavenging anti oxidant) is an important endogenousantioxidant enzyme act as the first line defence system against ROS which scavenges superoxide radicals to H_2O_2.
- Glutathione Peroxidase (GSH-P_X) present in the cytoplasm of the cells removes H_2O_2 by coupling its reduction to H_2O with oxidation of Glutathione (GSH). SH groups present in GSH to react with H_2O_2 and the OH• radical and prevent tissue damage andGSH is also capable of scavenging ROS directly or enzymatically via GSH-Px.
- Catalase is a water soluble enzyme. Catalase metabolize H_2O_2 to H_20 and O_2. GSH – P_X reduced both H_20_2 and organic hydro peroxides when reacting with GSH[42, 43].

Table No.: 2 Endogenous antioxidant defences

Antioxidant enzyme	Chemical name	Scavenged oxidant agent	General characters
GSH-PX	Glutathione Peroxidase	H202	It is major endogenous antioxidant molecule
SOD	Super oxide dismutase	O2.-	Necessary for the release of biological Active NO it protects NO from inactivation.
CAT	Catalase	H202	It catalyses the breakdown of H2O2 to H2Oand molecular oxygen.

Various antioxidants with different functions play their roles in the defense network, the free radical scavenging antioxidants being one of the players.Preventing antioxidant functions as first line defense by suppressing the formation ROS/RNS. Scavenging antioxidants remove active species rapidly by

the active species attack biologically essential molecules (SOD). Various enzyme functions by third line defence by repairing damages, clearing the wastes and reconstituting the lost function. Adaptation mechanism fourth line defence, in which appropriate antioxidants are generated at right time and transferred to right position right concentration[44].Exogenous antioxidants such as Vitamin E, Vitamin C, exist at a number of locations namely on the cell membrane, intracellular, extra cellular membrane. They react with ROS to either or remove or inhibit ROS. Fat soluble antioxidants like vitamin E protect against the loss of membrane integrity. Fat soluble antioxidants are important in preventing membrane PUFA from undamaging lipid peroxidation[44, 45].

ROLE OF FREE RADICALS IN PEPTIC ULCER[46]:

Peptic ulcers affect the gastrointestinal tract. Malignancies of the G.I. tract are relatively resistant to radiation therapy and chemotherapy has also had modest benefit, whereas an effective therapy still remains elusive in the treatment of gastroduodenal ulceration. One of the common cause of these diseases is the involvement of free radicals. Reactive oxygen species (ROS) are generated from numerous normal metabolic processes and are needed for normal functioning of the organism. Various antioxidant enzymes like SOD, GSH-P_X, and CAT control their accumulation. If there imbalance in the activity of these enzymes leads to formation of free radicals. These ROS are responsible for oxidation of DNA and causes tissue damage. Antioxidants seemed to have protective role in gastric ulcers and carcinomas[47].

II. AIM AND OBJECTIVE

AIM

The aim of this study is to evaluate the Antiulcer effect of *Echinops echinatus* Roxb.,on acetic acid and methylene blue induced ulcerations.

PROPOSED OBJECTIVE:

Herbs are the major contributors in the drug discovery which would be even cheap and safe. So we selected medicinal herbs. The plants were selected based on the ethno botanical claim. Based on the sequence of preliminary screening the total protocol was adopted for plants *Echinops Echinatus* Roxb., To get better components in the extract, ethanolic extraction was used.

III. REVIEW OF LITERATURE

Literature review of *Echinops Echinatus* Roxb.

i. **Sofia Eram et al., (2016)** carried out Experimental evaluation of *Echinops echinatus* as an effective hepatoprotective. Ethanolic extract of ariel parts of Echinops echinatus were evaluated on CCl4 induced liver damage in Rabbits, the extract at the dose level of 500 mg/kg is found to posses significant protection.

ii. **Manish Agrawal et al., (2012)** evaluated the protective effects of *Echinops echinatus* on testosterone-induced prostatic hyperplasia in rats*. Echinops echinatus* extracts attenuated the increase in the prostatic/body weight ratio induced by testosterone. Butanolic fraction of ethanolic extract at the dose level of 50 and 100 mg/kg exhibited the best activity.

iii. **Amish J. Patel et al., (2011**) performed Comparative diuretic activity of root and aerial part methanolic extracts of *Echinops echinatus* Roxb.Methanolic extracts of roots and aerial parts of *Echinops echinatus Roxb.* were subjected to diuretic potential in albino rats methanolic extracts at the dose of 250 and 500 mg/kg body weight shows a significant increase in the urine volume and electrolyte excretion when compared to control.

iv. **Leena S & Sitaram K (2010)** discussed Traditional uses of plants as cooling agents by the tribal and traditional communities of dang region in Rajasthan, India. *Echinops echinatus* Roxb. paste smeared on soles and palms to treat heatstroke.

v. **Rudrappa JN & Mohmoud R (2010)** studied Free radical scavenging activity of Echinops echinatus roxb Root. Extracts of Echinops echinatus Roxb, roots were evaluated for radical scavenging activities using different in vitro models like scavenging of 2, 2 diphenyl-1-picrylhydrazyl (DPPH) radical, nitric oxide radical and superoxide anion.

vi. **Singh S *et al*., (2006)** isolated Flavonoids from *Echinops echinatus*. A new isoflavone glycoside, echinoside (7), together with 7-hydroxyisoflavone, kaempferol-4'-methylether, kaempferol-7-methyl ether, myrecetin-3-O-alpha-L-rhamnoside, kaempferol and kaempferol-3-O-alpha-L-rhamnoside, has been isolated from the whole plant of *Echinops echinatus*.

vii. **Padashetty SA & Mishra SH (2005)** described antifertility activity of *Echinops echinatus* roots on male rats. The present study was undertaken to evaluate the effect of terpenoidal fraction prepared from the petroleum ether extract of the roots of *Echinops echinatus* on male reproductive parameters.

viii. **Khan MA *et al*., (2000)** discussed Ethnobotany and taxonomic studies of Echinops echinatus Roxb. (Untkatara) from Potohar region of Pakistan. The ethnobotanical information was verified by cross-checking with the people of different localities. The plant is diuretic, alterative, aphrodisiac and nervine tonic. It is also recommended in hysteria, dyspepsia, jaundice and scrofula.

ix. **Singh B *et al*., (1999)** described anti-inflammatory activity of ethanol extract of *Echinops echinatus* whole plant. The extract effectively inhibited the acute inflammation induced in rats by carrageenan, formaldehyde and adjuvant and the chronic arthritis induced by formaldehyde and adjuvant.

x. **Singh UP *et al*., (1998)** investigated antifungal activity of some new flavones and flavone glycosides of *echinops echinatus*. Four phenolic compounds, viz., apigenin, apigenin-7-O-glucoside, echinacin, and echinaticin, were isolated from the whole plant of *Echinops echinatus* Roxb.; the latter two compounds were isolated for the first time. Echinacin, which was highly effective at 150 µg mL−1, is considered the most promising of these compounds and its use as a control measure against *Alternaria* blight of pigeon pea under field conditions has been suggested.

xi. **Bupinder Sing *et al*., (1989)** investigated Anti-inflammatory activity of taraxasterol acetate from *Echinops echinatus* in rats and mice. Taraxasterol acetate, a triterpenoid found in several plant species, demonstrated antiinflammatory activity in albino rats against carrageenan, formaldehyde and adjuvant induced inflammations in doses between 10 and 100 mg/kg.

xii. **Chaudhuri et al., (1987)** isolated Echinozolinone, an alkaloid from *Echinops echinatus*. In addition to echinopsine and echinopsidine, a new alkaloid, echinozolinone, has been identified in *Echinops echinatus* as 3(2-hydroxyethyl)-4(3H)-quinazolinone from its spectral data.

IV. SCOPE AND PLAN OF WORK

SCOPE OF WORK

Gastric ulcer is one of the commonest diseases, Peptic ulcer disease is a serious gastrointestinal disease it requires a well targeted strategy. A number of drugs including proton pump inhibitors, prostaglandins analogs, histamine receptor antagonists and cytoprotective agents are available for the treatment of peptic ulcer. Reports on clinical evaluation of these drugs show that there are incidences of relapses and adverse effects and danger of drug interactions during ulcer therapy. This is been the rational for the development of new antiulcer drug and the search for novel molecules has been extended to herbal drugs that offer better protection and decreased relapse.

Several natural drugs have been reported on anti-ulcerogenic activity by virtue of their predominant effect on mucosal defensive factors. Plants and other natural substances has been used as the rich source of medicine. Ancient civilizations have documented medical uses of plant in their own ethnobotanical texts. The list of drugs obtained from different plant source is fairly extensive.

PLAN OF THE WORK

The following studies were carried out on ethanolic extract of *Echinops echinatus* Roxb.

i. **Collection and authentification of plant**.

ii. Preparation of ethanolic extracts and preliminary phytochemical screening of extracts.

iii. In-vitro antioxidant studies of the extracts.

a. Determination of DPPH radical scavenging activity.
b. Determination of nitric oxide scavenging activity.
c. Determination of hydroxyl radical scavenging activity.

iv. Toxicological investigation of the extracts.

a. Acute toxicity study.
b. Sub acute toxicity.

v. Antiulcer studies

a. Acetic acid induced gastric ulcer method

b. Methylene blue induced gastric ulcer method

V. MATERIALS AND METHODS

PLANT MATERIALS

The whole plant material for the plant *EchinopsEchinatus Roxb* were collected from the forest regions of tirupathi and were authenticated by Prof. P. Jayaraman, Director, National Institute of Herbal Science, Chennai. The Ref. no. PARC/-2018/043 respectively.

PLANT:

Scientific name	:	***EchinopsechinatusRoxb*.**
Family	:	Compositae / Asteraceae

Botanical Classification

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	*Echinops*
Sub class	:	Tracheobionta
Order	:	Yet to know
Genus	:	*Echinops*
Species	:	Echinatus

Vernacular Names

English	:	Indian Globe Thistle
Sanskrit	:	Brahmadanda
Tamil	:	Kutiraippijan
Hindi	:	Utakatira, Oontkateli, Gokhru
Kannada	:	Brahmadande
Telugu	:	Brahmadandi

Habitat

India - Andhra Pradesh, Bihar, Himachal Pradesh, Jammu and Kashmir, Karnataka, Madhya Pradesh, Maharashtra, Rajasthan, Tamil Nadu, Uttar Pradesh, West Bengal

Traditional Uses

The rural population of Gujarat uses the suspension of root bark powder for the treatment of diabetes. Chhattisgarh people use this herb in different ways both internally and externally for the treatment of sexual disorders. The patients suffering from respiratory troubles, particularly asthma, are advised to inhale the

fumes obtained by burning the leaves & roots of *E. echinatus* in order to get quick and permanent relief. The root is abortifacient aphrodisiac. The seeds are sweet and aphrodisiac. The plant is bitter, stomachic, antipyretic, analgesic, increases the appetite stimulates the liver, useful in brain disease, used in ophthalmia, chronic fever, pains in the joints, inflammations, the root is aphrodisiac.

EXPERIMENTAL ANIMALS

Albino wistar rats of either sex approximately same age group were used after being acclimatized for a week at laboratory conditions. They were provided standard rodent pellet diet (Lipton India) and water *ad libitum*. The animals had free access to food and water and maintained under 12:12 hr light and dark cycle. All experiments were carried out during day time. The protocol was approved by institutional animal ethical committee and care of the animals was taken as per guidelines of committee for the purpose of control and supervision in experiments on animals (CPCSEA), representative of animal welfare, Govt of India.

The experimental protocol was approved by the Institutional Animal ethics Committee IAEC Ref. No. SVCE/BIO/2018/011

CHEMICALS:

S.No	Chemicals	Source
1	Acetic acid	Sigma chemical company, USA
2	Acetyl thiocholine iodide	S.d.fine chemicals Ltd.,Mumbai
3	Adenosine 5’ diphosphate	S.d.fine chemicals Ltd.,Mumbai
4	Chloroform	S.d.fine chemicals Ltd., Mumbai
5	Disodium hydrogen phosphate	S.d.fine chemicals Ltd., Mumbai
6	5,5’-Dithio-bis-2-nitrobenzoic acid(DTNB)	Loba chemicals Ltd, Mumbai.
7	Disodium dihydrogen phosphate.	S.d.fine chemicals Ltd.,Mumbai
8	Ethyl Acetate	S.d.fine chemicals Ltd.,Mumbai
9	Ethylene diamine tetra acetic ac (EDTA) Disodium. Salt	Rolex lab reagent, Mumbai.
10	FeCl3 reagent	S.d.fine chemicals Ltd., Mumbai
11	Glycine	S.d.fine chemicals Ltd., Mumbai
12	Glutamate dehydrogenase	S.d.fine chemicals Ltd., Mumbai
13	Hydrogen peroxide	Qualigens fine chemicals Ltd Mumbai
14	Hydrazine hydrate	S.d.fine chemicals Ltd..Mumbai
15	Iodine	S.d.fine chemicals Ltd.,Mumbai
16	Methanol (analytical grade)	S.d.fine chemicals Ltd., Mumbai
17	n-butanol	S.d.fine chemicals Ltd.,Mumbai

18	NAD (Nicotinamide adenine dinucleotide)	S.d.fine chemicals Ltd., aiMumb
19	NADPH2	S.d.fine chemicals Ltd., Mumbai
20	Oxidised gluthathione.	Qualigens fine chemicals Ltd., Mumbai.
21	Potassium dihydrogen phosphate	S.d.fine chemicals Ltd., Mumbai
22	Pthalyl dialdehyde.	S.d.fine chemicals Ltd., Mumbai
23	Perchloric acid	Loba chemicals Ltd, Mumbai.
24	Pyrogallol	S.d.fine chemicals Ltd., Mumbai
25	Reduced Glutathione (GSH)	S.d.fine chemicals Ltd., Mumbai
26	Sodium dodecyl sulphate(SDS)	S.d.fine chemicals Ltd., Mumbai
27	Sodium Acetate	S.d.fine chemicals Ltd., Mumbai
28	Sodium hydrogen carbonate	S.d.fine chemicals Ltd., Mumbai
29	Thionyl chloride.	S.d.fine chemicals Ltd., Mumbai
30	Trichloro acetic acid	Ranbaxy laboratories Ltd. Chemical division, New delhi.
31	Tris HCl	S.d.fine chemicals Ltd., Mumbai
32	Thiobarbituric acid	Rolex laboratories reagent, Mumbai

Extraction and Identification of Phytoconstituents

Extraction of plant material (*Harbone 1973***)**

The air dried parts of *Echinops Echinatus Roxb*., 500gms each were coarse powdered and extracted with 50% alcohol. The crude extract was further filtered and evaporated by the aid of rotary evaporator. The final mass is weighed and preserved for further use.

Preliminary phytochemical screening:

Preliminary phytoconstituents present in the hydroalcholic extract of *Echinops Echinatus* plants were identified based on the chemical test. *(Kokate CK1994)*

1. Test for alkaloids

Treated with dilute Hydrochloric acid and filtered. The filtrate was treated with various alkaloidal agents.

a) Mayer's–Test

Treated with Mayer's reagent and cream colour indicates the presence of alkaloid.

b) Dragendroff's–Test

When little amount of the sample was treated with the Dragendroff's reagent, the presence of reddish brown precipitate reveals the presence of alkaloid.

c) Hager's–Test

Treated with the Hager's reagent and presence of yellow colour precipitate indicates the presence of alkaloid.

d) Wagner's–Test

Treated with the Wagner's reagent, the appearance of brown colour precipitate indicates the presence of alkaloid.

2. Test for carbohydrates

The extracts were treated with 3ml of alpha–Napthol in alcohol and to the sides of the test tube concentrated sulphuric acid was added carefully. Formation of violet colour ring at the junction of two liquids shows the presence of carbohydrates.

a) Fehling's–Test

The extracts were treated with Fehling's solution A and B and heated. Presence of reddish brown colour precipitate indicates the presence of reducing sugars.

b) Benedict's–Test

The extracts were treated with Benedict's reagent and heated and presence of reddish orange colour precipitate indicates the presence of reducing sugars.

c) Barfoed's–Test

The extracts were treated with Barfoed's reagent and heated. Appearance of reddish orange colour precipitate indicates the presence of non reducing sugars.

3. Test for proteins

a) Biuret's–test

When the extracts were treated with copper sulphate solution, followed by the addition of sodium hydroxide solution, appearance of violet colour indicates the presence of proteins

b) Millon's–Test

When the extract was treated with Millon's reagent, appearance of pink colour indicates the presence of proteins.

4. Test for steroids

a) Libermann Burchard Test

When the extracts were treated with concentrated sulphuric acid, few drops of glacial acetic acid, followed by the addition of acetic anhydride, appearance of green colour indicates the presence of steroids.

5. Test for sterols

When the extracts were treated with 5% potassium hydroxide solution, appearance of pink colour indicates the presence of sterols.

6. Test for phenols

When the extracts were treated with neutral ferric chloride solution, appearance of violet colour indicates the presence of phenols.

When the extracts were treated with 10% sodium chloride solution, the appearance of cream colour indicates the presence of phenols.

7. Test for tannins

a) When the extracts were treated with 10% lead acetate solution, appearance of white precipitate indicates the presence of tannins.
b) When the extracts were treated with aqueous bromine solution, appearance of white precipitateindicates the presence of tannins.

8. Test for flavanoids

a) 5ml of the extract solution was hydrolyzed with 10 % v/v sulphuric acid and cooled. Then, it was extracted with diethyl ether and divided into three portions in three separate test tubes. 1 ml of diluted sodium carbonate, 1 ml of 0.1N sodium hydroxide, and 1ml of strong ammonia solution were added to the first, second and third test tubes respectively. In each test tube, development of yellow color demonstrated the presence of flavonoids.

b) Shinoda's test

The extract was dissolved in alcohol, to that one piece of magnesium followed by concentrated HCl was added drop wise and heated. Appearance of magenta color shows the presence of flavonoids.

9. Test for gums and mucilage

The extracts were treated with 25 ml of absolute alcohol, and then solution was filtered. The filtrate was examined for its swelling properties.

10. Test for glycosides

When a pinch of the extracts were dissolved in the glacial acetic acid and few drops of ferric chloride solution was added, followed by the addition of concentrated sulphuric acid, formation of red ring at the junction of two liquids indicates the presence of glycosides.

11. Test for saponins

Foam test

1ml of the extracts are diluted to 20 ml with distilled water and shaken well in a test tube. The formation of foam in the upper part of the test tube indicates the presence of saponins.

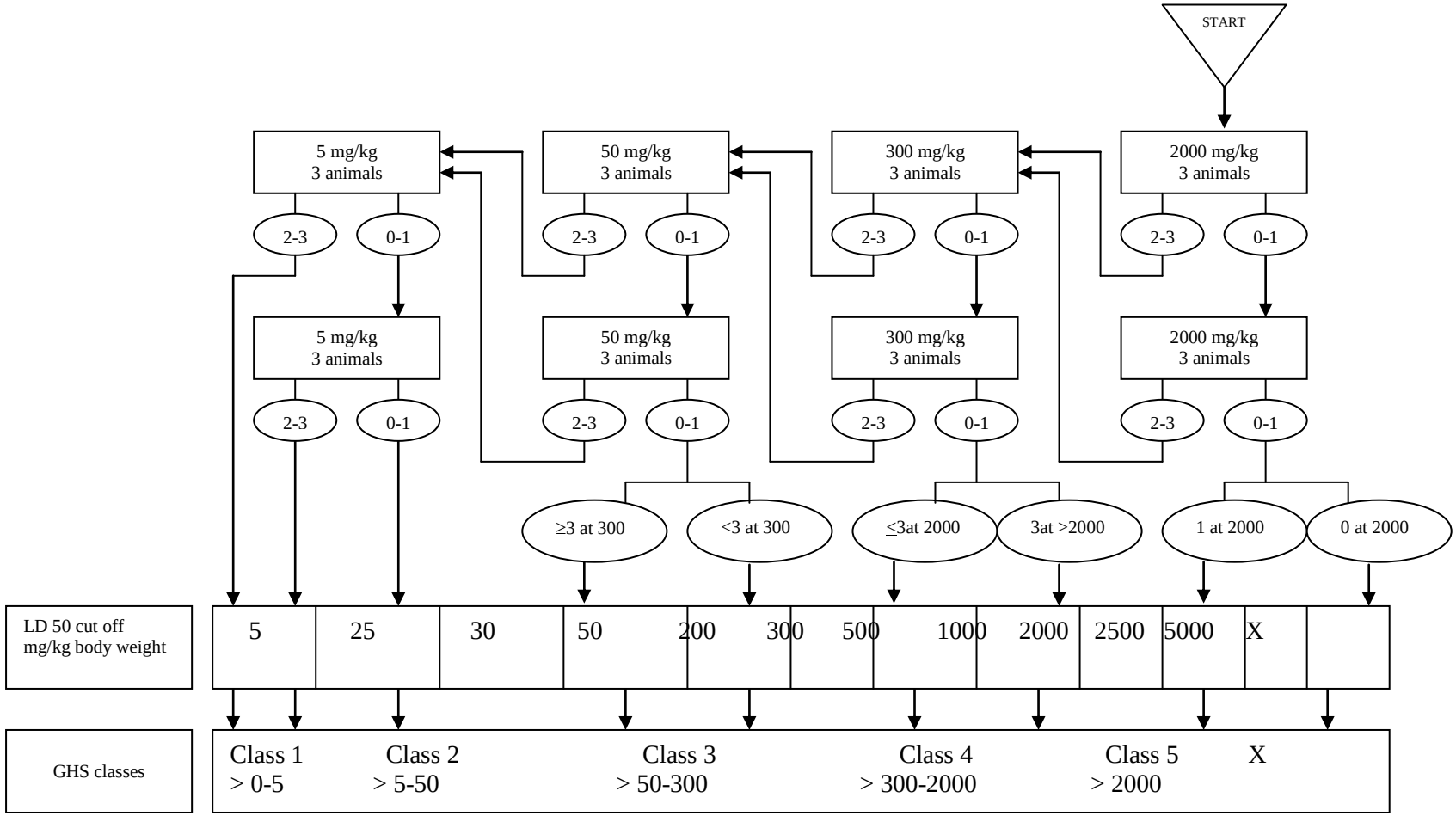
5.6 TOXICITY STUDY

5.6.1 Acute oral toxicity study (OECD 423)[50]

For carrying out oral toxicity study OECD guidelines 423 was followed. It is a stepwise procedure with three animals of a single sex per step. Depending on the mortality and/or, 50, 300, 2000mg/kg body weight and the results allow a substance to be ranked morbidity of the animals a few steps may be necessary to judge the toxicity of the test substance. This procedure has advantage over other methods because of minimal usage of animals while allowing for acceptable data.

The method uses defined doses (5and classified according to the globally harmonized system. The starting dose for ethanolic extract was 2000mg/kg bodyweight (p.o). The dose was administered to the rats which were fasted overnight with water *ad libitum* and observed for signs of toxicity. The same dose was once again tried with another three rats and were observed for 72 hours for symptoms like change in skin colour, salivation, diarrhea, sleep, tremors, convulsions and also respiratory, autonomic and CNS effects.

Fig-3 Flow Chart for acute toxic class method (OECD guidelines 423) at starting dose of 2000 mg/kg body weight/p.o



0,1,2,3: Number of moribund or dead animals at each step
GHS: Globally Harmonized classified system (mg/kg b.w) X-Un classified

SUB ACUTE TOXICITY STUDY

For carrying out sub acute oral toxicity study OECD guidelines 407 -Repeated Dose 28-Day oral toxicity study in rodents was followed. The duration of the Study was 28 days. Three dose levels of the plant extract were used (200 & 400 mg/kg). Each group consists of ten animals. The drug was administered orally once daily for 28 days. On 29th day the animals was anaesthetized and blood was collected by retro orbital puncture. Hematological parameters were evaluated. Serum was separated and biochemical parameters were estimated. Animals were sacrificed and organs were removed and weighed. The organs were kept in 10% formalin and used for histo-pathological analysis.

Change in body weight

The change in body weight was observed for a time period of 28 days at an interval of 7 days. Body weight gains were determined from the final and initial body weights[52]

Organ weight

After sacrifice, organs were quickly excised and weighed (absolute organ weight and relative organ weights were computed. ((Liver weight : Body weight) x 100)

HAEMATOLOGICAL STUDIES

The following haematological parameters were estimated by standard procedures.

Total R.B.C. count

The enumeration of red blood corpuscles was carried out on blood samples using haemocytometer as per standard methods. The blood was taken in the R.B.C. pipette upto 0.5 marks and diluted with R.B.C. diluting fluid (Hyme's fluid) upto 101 marks. It was mixed well and mounted on counting chamber. The red blood cells were then counted using a haemocytometer and their number in undiluted blood was calculated. R.B.C. count in blood is expressed as cells in millions/mm3.[58]

Total W.B.C. Count

The blood was taken in W.B.C. pipette upto 0.5 mark and diluted with W.B.C. diluting fluid (1-2% acetic acid stained lightly with gentian violet), where acetic acid

destroy the red blood corpuscles and gentian violet stains the nuclei of white blood cells. The fluid in the pipette was mixed well and mounted in the counting chamber. The W.B.C. count was then counted and their number in undiluted blood was calculated. W.B.C. count in blood is expressed as cells in 1000/mm3.[61]

Differential leukocyte count

A blood film stained with Leishman's stain was examined under oil immersion and the different types of blood cells were identified. The percentage distribution of these cells was then determined as follows:

A thin blood film was prepared, dried and placed on the staining rack and was covered with Leishman's stain and allowed to stand for 2mins. Then equal volumes of distilled water was added and mixed by tilting the slide first-one way and then the other way. It was allowed to stand for 6mins, drained off diluted stain in a stream of distilled water from a wash bottle for about 20 seconds and allowed the slide to remain on the rack for 1-2mins with the last wash covering it. The slide was kept against a support in an inclining position, stained the smear facing down and allowed to dry.

Then the stained slides were studied under low and high power objectives for differential counts by placing two drops of cedar wood oil on the stained smear and using oil-immersion lens. The differential count is expressed as percentage for neutrophils, lymphocytes, eosinophils, monocytes and basophils.[63]

Haemoglobin (Hb) concentration

Haemoglobin in the red blood corpuscles was converted to acid haematin by adding N/10 hydrochloric acid. The brown colour developed was matched against standard brown tinted glass in the comparator by direct vision. The haemoglobin concentration in blood is expressed as grams per 100 ml of blood (g%).[65]

BIOCHEMICAL STUDIES:

Blood samples were drawn by cardiac puncture. Blood from three animals was pooled for serum separation. Each serum sample was analyzed by auto analyzer

i. Aspartate Aminotransferase (ASAT)
ii. Alanine Aminotransferase (ALAT)
iii. Alkaline Phosphatase (ALP)
iv. Total Bilirubin (TB)

v. Direct Bilirubin (DB)
vi. Urea
vii. Creatinine
viii. Total protein.

HISTOPATHOLOGICAL STUDY

After blood collection rats were sacrificed for histopathology studies. The internal organs like liver, kidney, lungs, brain, heart and spleen were isolated and blotted free of blood, weighed immediately to determine relative organs weight and observed for gross lesions. Histological examination was performed on the tissue preserved in 10% buffered formalin solution with particular emphasis on those which showed gross pathological changes.[67]

Liver and kidneys were evaluated for histopathological changes. After collection of organs, the tissues were stored in 10% formalin for 48 hrs. The solution was changed after 24hrs. In the case of kidneys, two incomplete parallel cuts were made from cortex to pelvis and one longitudinal cut was made which divides the kidney into two halves. In case of liver one piece from each lobe was used. The tissues were then wax embedded as below:

Solvent	**Duration** (Holding Period)
50% Alcohol	30 minutes
70% Alcohol	30 minutes
95% Alcohol	20 minutes
95% Alcohol	20 minutes
100% Alcohol	30 minutes
100% Alcohol	30 minutes
Chloroform	30 minutes
Chloroform	30 minutes
Wax	10 minutes

They were then blocked in wax and allowed to set on a cold plate. Once cooled they were cut into 5µ size sections. The sections were kept in slides and stained with haematoxyline and eosin.

EVALUATION OF ANTI ULCER ACTIVITY

ACETIC ACID INDUCED ULCERS

Rats were divided in to 4 groups (n=6) and the duration of the experiment was 7 days.

Groups	Treatment
Group-I	Rats received normal distilled water for 7 days.
Group-II	Rats received Omeprazole 20mg/kg with dilute acetic acid (4%) (2 ml) submucosal injection after the 7th day
Group –III	Rats received 200 mg/kg EE oral with dilute acetic acid (4%) (2 ml) submucosal injection after the 7th day
Group –IV	Rats received 400 mg/kg EE oral with dilute acetic acid (4%) (2 ml) submucosal injection after the 7th day

At the end of the treatment period, rats were deprived of food overnight and sacrificed on day 8th day by light ether anesthesia followed by decapitation after recording the final body weight. Blood was collected from each rat for biochemical estimation and stomach along with duodenum was quickly isolated immersed in ice cold saline and weighed.

Acetic Acid-Induced Gastric Ulcers

Gastric ulcer induced in rats by means of submucosal injection of acetic acid and reported on the healing process of lesions for extended intervals after the ulcer preparation.[20]

The procedure for inducing ulcers by acetic acid involves fasting rats for 24–36 hours with access to water *ad libitum*. The animals are observed to ensure good health before induction of ulcers. First, the animals are put under light ether anesthesia. A flexible plastic catheter with an outside diameter of 2 mm is inserted 8 cm into the

colon via the anus through which dilute acetic acid (4%) (2 ml) is introduced into the colon. The rats are then maintained in a head-down position for 2 minutes to prevent leakage of the acetic acid solution. After 24 hours of acetic acid administration, the animals are started the treatment with ethanolic extract of *Echinops echinatusRoxb*. [61]. It is prudent to conduct a preliminary dose-finding study to determine optimum dose for ulcer induction.

METHYLENE BLUE-INDUCED ULCER

To induce ulcers with MB, animals are fasted 24 hours before MB administration at a dosage of 125 mg/kg body weight p.o. followed by the administration of the drug(s) or substances under investigation. Animals are sacrificed after 4 hours of MB administration, and ulcer index determined

Rats were divided in to 4 groups (n=6) and the duration of the experiment was 7 days.

Groups	Treatment
Group-I	Rats received normal distilled water for 7 days.
Group-II	Rats received Omeprazole 20 mg/kg with MB administration at a dosag of 125 mg/kg after the 7th day
Group –III	Rats received 200 mg/kg EE oral with MB administration at a dosage of 125 mg/kg after the 7th day
Group –IV	Rats received 400 mg/kg EE oral with MB administration at a dosage of 125 mg/kg after the 7th day

At the end of the treatment period, rats were deprived of food overnight and sacrificed on day

Ulcer Scoring

After sacrificing the rat, stomach was removed and opened along the greater curvature, and washed it slowly under running tap water. Put it on the glass slide and observe

under 10X magnification for ulcer. Score the ulcers as below. 0= normal coloured stomach

0 = no damage

1 = blood at the lumen

2 = pinpoint erosions

3 = one to five small erosions <2 mm,

4 = more than five small erosions <2 mm,

5 = one to three large erosions >2 mm,

6 = more than three large erosions >2 mm

Mean ulcer score for each animal is expressed as Ulcer Index.

Free acidity and Total acidity.

One ml of gastric juice is pipette in to 100ml conical flask and titrate with 0.01N NaOH using topfers reagent as an indicator. (It is Dimethyl-amino-azo-benzene with phenolphthalein and used for detection and estimation of hydrochloric acid and total acidity in gastric fluids) Titrate to end point when the solution turns to orange colour. Note the volume of NaOH which corresponds to free acidity. Titrate further till the solution regains its pink colour. Note the total volume of NaOH which corresponds to the total acidity. Acidity (mEq/L/100 g) can be expressed as

$$\text{Acidity} = \frac{volume of NaoH \times Normality of Na}{100} \times 100$$

INVITRO ANTI-OXIDANT ACTIVITY

DPPH Radical Scavenging Assay([78]):

The DPPH free radical is reduced to a corresponding hydrazine when it reacts with hydrogen donors. The DPPH radical is purple in colour and upon reaction with hydrogen donor changes to yellow colour. It is a discoloration assay, which is evaluated by the addition of the antioxidant to a DPPH solution in ethanol or methanol and the decrease in absorbance was measured at 490nm.

Reagents: 2, 2-Diphenly 1-picryl hydrazyl solution (DPPH, 100 M): 22 mg of DPPH was accurately weighed and dissolved in 100 ml of methanol. From this stock solution, 18 ml was taken and diluted to 100 ml using methanol to obtain 100 M DPPH solution.

Preparation of test solution: 21mg each of the extract was dissolved in distilled DMSO separately to obtain solutions of 21 mg/ml concentrations. Each of these solutions was serially diluted separately to obtain lower concentrations

Preparation of standard solutions: 10 mg each of ascorbic acid and rutin were weighed separately and dissolved in 0.95 ml of Dimethyl sulfoxide (DMSO) to get 10.5 mg/ml concentrations. These solutions were serially diluted with DMSO to get lower concentrations.

Procedure: The assay was carried out in a 96 well microtitre plate. To 200 l of DPPH solution, 10 Ml of each of the test sample or the standard solution was added separately in wells of the microtitre plate. The final concentration of the test and standard solutions used were 1000, 500, 250, 125, 62.5, 31.25 and 15.625, 7.812 □ g/ml. The plates were incubated at 37o C for 30 min and the absorbance of each solution was measured at 490 nm, using a micro plate reader.

$$\% \text{ of inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance Test}}{\text{Absorbance of control}} X\ 100$$

SCAVENGING OF NITRIC OXIDE RADICAL:

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be estimated by the use of modified Griess Ilosvay reaction. In the present investigation, Griess Ilosvay reagent is modified by using Naphthyl ethylene Diamine Dihydro chloride (0.1% w/v) instead of 1-napthylamine (5%). Nitrite ions react with Griess reagent, which forms a purple azo dye. In presence of test components, likely to be scavengers, the amount of nitrites will decrease. The degree of decrease in the formation of purple azo dye will reflect the extent of scavenging. The absorbance of the chromophore formed was measured at 540 nm[79, 80].

a) Reagents:

Sodium nitroprusside solution: Weighed accurately 0.2998 g of sodium nitroprusside and dissolved in distilled water to make up the volume to 100 ml in a volumetric flask (10 mM).

Naphthyl Ethylene Diamine Di hydrochloride (NEDD, 0.1%):

Weighed accurately 0.1 g of NEDD and dissolved in 60 ml of 50% glacial acetic acid by heating and made up the volume to 100 ml in a volumetric flask with distilled water.

Sulphanilic acid (0.33% w/v) reagent:

Weighed accurately 0.33 g of sulphanilic acid and dissolved in 20% glacial acetic acid by heating and made up the volume to 100 ml in a volumetric flask.

Preparation of test sample: The sample solution was prepared as described in DPPH assay.

Preparation of standard solutions:

Weighed accurately 10 mg of ascorbic acid and rutin and dissolved in 1 ml of DMSO separately. From these solutions, serial dilutions were made to obtain lower concentrations using DMSO.

Procedure: The reaction mixture (6 ml) containing sodium nitroprusside (10 mM, 4 ml), phosphate buffer saline (PBS, pH 7.4, 1 ml) and extract in DMSO at various concentrations or standard was incubated at 25o C for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrite ion was removed, 1 ml of sulphanilic acid reagent was added, mixed well and allowed to stand for 5 min for completion of diazotization. Then, 1 ml of NEDD was added, mixed and allowed to stand for 30 min in diffused light. A pink coloured chromophore was formed. The absorbance of these solutions was measured at 540 nm.

$$\% \text{ of inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance Test}}{\text{Absorbance of Control}} \times 100$$

VI. RESULTS

Percentage Yield of Leaf Extract:

The yield obtained from the ethanolic extract of *EchinopsEchinatus* Roxb was about 12gms from 500gms of dried plant.

The percentage yield of extract = 2.4%

PRELIMINARY PHYTOCHEMICAL STUDIES:

The Results of preliminary Phytochemical Screening of the ethanolic extract of *EchinopsEchinatus* Roxb were given in Table 3. It shows presence of

Alkaloid, Flavonoids, Glycosides, Phytosterols, Triterpenoids, Tannins and Proteins

Sl.No	Test	EE
1	Test for carbohydrates Molisch's test	Absent
2	Test for Alkaloids Mayer's test Dragendrodroff's test	Present
3	Test for Flavonoids Alkaline reagent test	Present
4	Test for Glycosides Modified Borntrager's test Keller- Killiani test	Present
5	Leiberman-Bucharat test Salkowaski test	Present
6	Test for Tannins	Present
7	Test for Proteins	Present

Table: 3. Preliminary Phytochemical Screening of the ethanolic extract of *Echinopsechinatus* Roxb

ACUTE TOXICITY STUDIES

The treatment of animals with Ethanolic extracts of *EchinopsEchinatus* has not shown any changes in autonomic or behavioural responses in rats. There is no mortality upto 2000mg/kg body weight.

S. No	Parameters	Result
1	Motor activity	Absent
2	Tremors	Absent
3	Convulsion	Absent
4	Straub reaction	Absent
5	Pilo erection	Absent
6	Loss of lighting effects	Absent
7	Sedation	Absent
8	Muscle relaxation	Absent
9	Hypnosis	Absent
10	Analgesia	Absent
11	Ptosis	Absent
12	Lacrimation	Absent
13	Diarrhoea	Absent
14	Change in skin colour	No change

Table no: 4 observation on acute toxicity studies at the dose of 2000mg/kg

Parameters observed	Day-	Day-	Day-	Day-	Day-	Day-	Day-7	Day-	Day-	Day-1	Day-1	Day-1	Day-1	Day-14
Aggressiveness	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alertness	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alopecia	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Circling	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diarrhoea	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Edema	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Eye closure at touc	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Grip strength	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Grooming	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lacrimation	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of writing refle	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mortality	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nasal sniffing	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Piloerection	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rearing	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Righting reflex	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Seizures	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Straub tail	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Urine stains	-	-	-	-	-	-	-	-	-	-	-	-	-	-

6.3.2 Observation in acute toxicity studies at the dose of 2000mg/kg bw p.o dose of *Anisomeles malabarica* (L.)

I. SUB-ACUTE TOXICITY STUDIES

Sub-acute toxicity studies were carried out according to OECD 407 and rats were divided into groups of 10 animals (5 male and 5 female). The suspension of ethanolic extract was administered to rats at the dose of 100 & 200 mg/kg/day for 28 days. The toxic symptoms such as signs of toxicity, mortality and body weight changes were monitored. Rats were anesthetized with ether at the end of the treatment period. All rats were sacrificed after the blood collection.

Parameters observed	Day-2	Day-4	Day-6	Day-8	Day-10	Day-12	Day-14	Day-16	Day-18	Day-20	Day-22	Day-24	Day-26	Day-28
Aggressiveness	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alertness	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alopecia	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Circling	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diarrhoea	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Edema	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Eye closure at touch	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Grip strength	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Grooming	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lacrimation	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of writing reflex	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mortality	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nasal sniffing	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Piloerection	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rearing	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Righting reflex	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Seizures	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Straub tail	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Urine stains	-	-	-	-	-	-	-	-	-	-	-	-	-	-

6.3.3 Signs of toxicity in sub acute toxicity (28 days)

CHANGE IN BODY WEIGHT

Treatment	0th day	5th day	10th day	15st day	20th day	25th day	28th day	% increase
Control	175.83±6.84	179.50±6.28	181.83±6.46	184.83±6.31	187.16±6.01	190.66±6.46	193.66±5.70	10.3637
100mg/kg	177.00±4.43	180.50±4.47	182.83±5.02	186.16±5.40	190.00±6.04	192.500±5.70	195.500±5.63	10.1695
200mg/kg	177.16±8.02	178.83±8.17	182.83±8.23	186.00±7.85	189.000±8.11	192.16±8.43	194.83±8.34	09.6044

6.3.4 Change in body weight after the drug treatment *EchinopsEchinatus* Roxb

6.3.5 Haematological Parameter

Haematological parameter	Control	*EchinopsEchinatus* Roxb	
		200 mg	400mg
Total R.B.C. count (×10^6 mm−3).	9.09±0.15	8.90±0.12	9.11±0.16
Total W.B.C. Count (×10^3 mm−3).	12.67±0.22	12.35±0.15	11.23±0.23
Haemoglobin (Hb) (g/dl)	15.61±0.36	14.07±0.30	15.63±0.36
Hematocrit (%).	44.21±1.01	43.61± 1.72	36.4±1.36
Platelets (×103 mm−3).	834.91±24.01	867.21±23.25	739.81±26.86
Lymphocytes(%).	84.7±1.32	81.8±1.33	72.8±1.43
Neutrophils (%).	20.6±0.65	12.6±0.52	19.2±0.91

Data are expressed as mean ± SEM

6.3.6 Biochemical Parameters

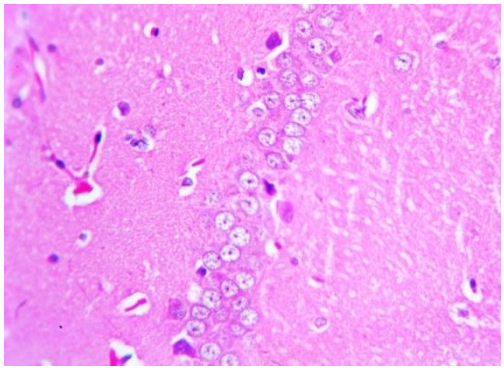
Biochemical parameter	Control	*EchinopsEchinatus* Roxb	
		200 mg	400mg
Creatinine (mg/dl)	0.5890±0.079	0.6600 ± 0.049	0.5540 ± 0.074
Urea (mg/dl)	15.30 ± 0.47	14.50 ± 0.40	15.20 ±0.57
Triglycerides (mg/dl)	52.20±1.13	51.40±1.08	47.10±1.62
Total Cholesterol (mg/dl)	46.60±1.21	51.40±1.08	54.03±1.67
Total protein (mg/dl)	4.40±0.26	4.20±0.35	3.70±0.26
Albumin (g/dl)	3.20±0.41	3.70±0.33	3.20±0.29
AST (IU/L)	121.41±2.68	121.3±1.65	116.61±2.045
ALT (IU/L)	69.40±1.57	67.60±1.301	68.60±1.108
ALP (IU/L)	112.6±4.67	117.01±0.714	117.41±0.718
T. Bilirubin (mg/dl)	0.2569±0.32	0.267±0.029	0.254±0.023

Data are expressed as mean ± SEM

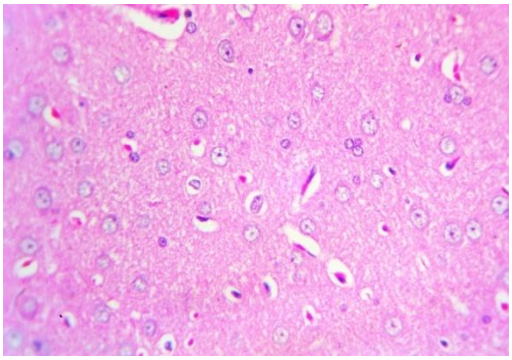
6.4 HISTOPATHOLOGY OF VITAL ORGANS

At the end of the 30th day Liver, Kidney, Heart, Lungs and Brain were subjected for routine Histopathological examination and fixed in 10 % formal saline (10 parts of formaldehyde and 30 parts of normal saline). Tissues were processed and embedded in paraffin wax. Sections were cut at 5 micron thickness and stained with Haematoxylin and Eosin. Light microscopic examination of the sections was then carried out and micrographs produced using Vanox-T Olympus photographing microscope. The histopathological examinations were reviewed by the pathologist.[60]

BRAIN

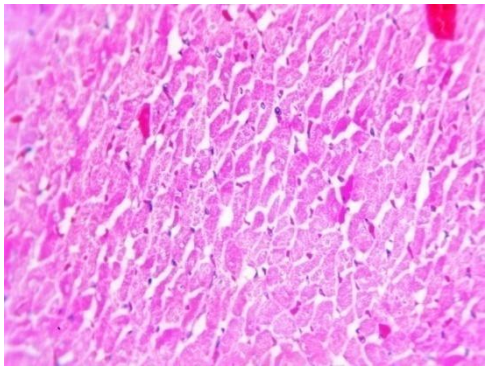


200mg/kg

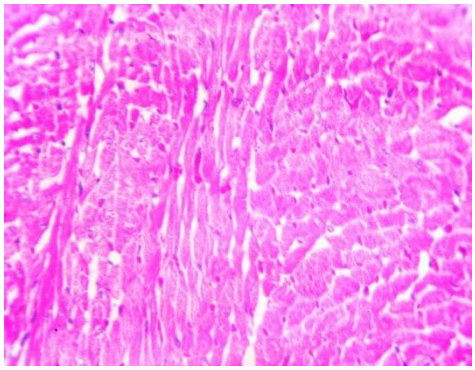


400mg/kg

HEART

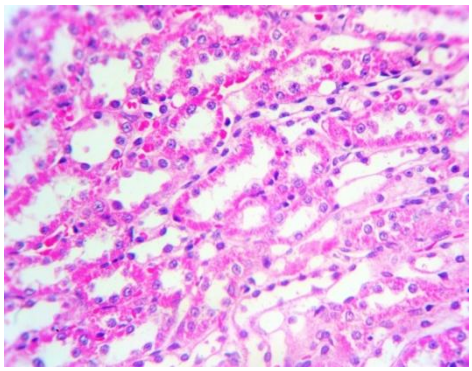


200mg/kg

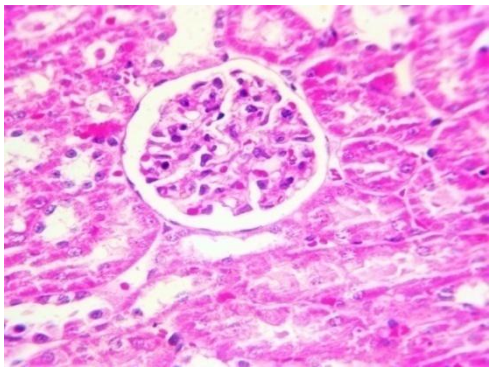


400mg/kg

KIDNEY

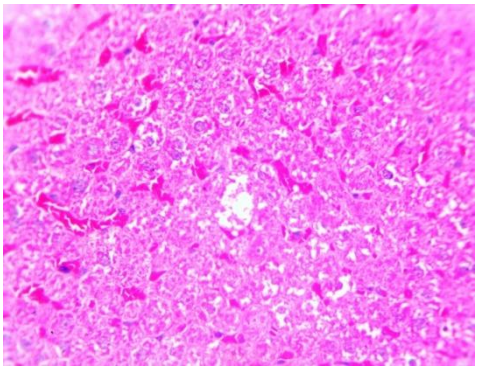


200mg/kg

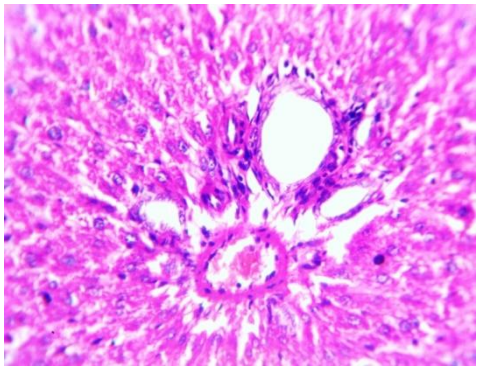


400mg/kg

LIVER

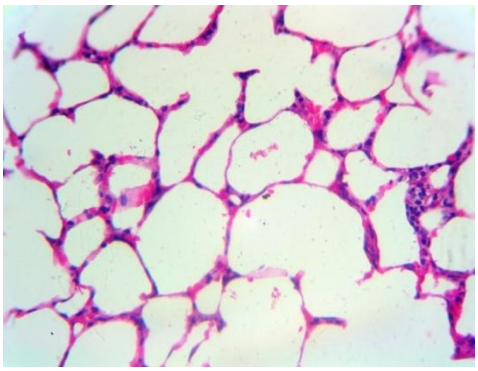


200mg/kg

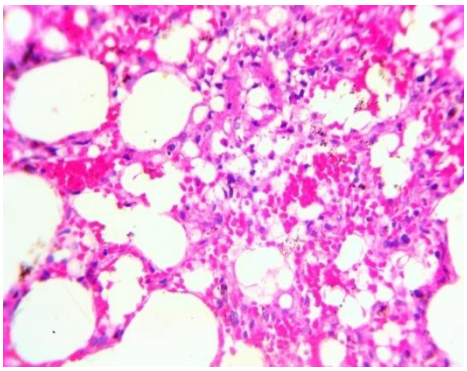


400mg/kg

LUNG



200mg/kg



400mg/kg

ANTI-ULCER ACTIVITY- Acetic Acid Induced ulcerations

Effect of Gastric Volume:

Group No.	Body wt. gms	Treatment	Vol. of gastric Juice	pH
	180		4.5ml	2
	165		5.0ml	2
	175	Control	4.6ml	2
	250		4.2ml	3
	210		5.2ml	2
	205		4.2ml	2
I			4.61 ±0.153	2.16 ± 0.152
	165		2.0ml	5
	185	dilute acetic acid (4%)	1.8ml	7
	210	(2 mL)	1.6ml	6
	175	&	2.2ml	6
	205	Omeprazole(20mg/kg)	2.8ml	7
	195		1.9ml	5
II			2.05 ±0.155	6 ± 0.333
	200		3.2ml	4
	195	dilute acetic acid (4%)	3.4ml	4
	180	(2 mL)	2.8ml	4
	165	&	3.5ml	5
	210	Ethanolic	3.2ml	4
	215	extract(200mg/kg)	3.0ml	3
III			3.18 ±0.104	4.0 ± 0.258
	195		2.8ml	5
	185	dilute acetic acid (4%)	2.4ml	6
	220	(2 mL)	2.2ml	5
	215	&	2.4ml	5
	185	Ethanloic extract	2.2ml	6
	175	(400mg/kg)	2.6ml	6
IV			2.43 ±0.09*	5.5 ± 0.204*

Table: 5. Effect of EE on Gastric Volume and pH in Acetic acid Induced Ulcer

Values are expressed in terms of mean ± SEM of 6 rats (ANOVA)
*Statistically significant P ≤0.05 (in comparison with Standard).

Effect of Free Acidity and Total Acidity:

Group No.	Body wt. gms	Treatment	Free acidity	Total acidity
I	180 165 175 250 210 205	Control	24 20 22 19 24 22 21.83 ± 0.833	42 43 40 39 44 42 41.66 ± 0.760
II	165 185 210 175 205 195	dilute acetic acid (4%) (2 mL) & Omeprazole(20mg/kg)	12 10 8 12 9 11 10.33 ± 0.66	24 21 19 16 21 19 20 ± 1.09
III	200 195 180 165 210 215	dilute acetic acid (4%) (2 mL) & Ethanolic extract(200mg/kg)	14 16 18 17 18 16 16.5 ± 0.61	32 31 35 28 34 30 31.66 ± 1.05
IV	195 185 220 215 185 175	dilute acetic acid (4%) (2 mL) & Ethanloic extract (400mg/kg)	12 14 12 12 10 11 11.8 ± 0.54*	21 24 22 25 26 22 23.33 ± 0.80*

Table: 6. Effect of EECP on Free Acidity and Total Acidity in Acetic acid Induced Ulcer:

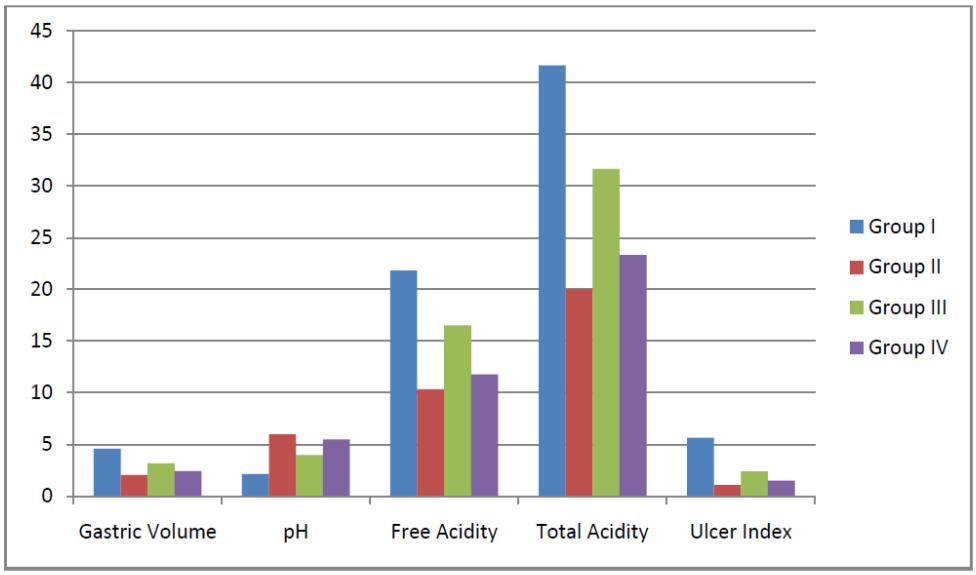
Values are expressed in terms of mean ± SEM of 6 rats (ANOVA)
*Statistically significant P ≤0.05 (in comparison with Standard).

Ulcer index:

Group No	Body Weight gm	ULCER INDEX						Total Score	Mean Ulcer Index ± SEM	% Protection
		Normal Colour stomach	Normal Colour stomach	Ulcer Spots	Hemorrh-agic streaks	Ulcers ≥3 but ≤5	Ulcer >5			
	180	-	0.5	1	1.5	-	3	6		
	165	-	0.5	1	1.5	2	-	5		
	175	-	0.5	1	1.5	-	3	6		
	250	-	0.5	1	1.5	2	-	5	5.666	
	210	-	0.5	1	1.5	-	3	6	±0.192	
I	205	-	0.5	1	1.5	-	3	6	45	-
	165	-	0.5	1	-	-	-	1.5		
	185	-	0.5	-	-	-	-	0.5		
	210	-	0.5	1	-	-	-	1.5		
	175	-	0.5	-	-	-	-	0.5	1.083	
	205	-	0.5	-	-	-	-	0.5	±0.247	
II	195	-	0.5	-	1.5	-	-	2	6	77.5
	200	-	0.5	-	1.5		-	2		
	195	-	0.5	1	1.5	-	-	3		
	180	-	0.5	1	-	-	-	1.5		
	165	-	0.5	1	1.5	-	-	3		
	210	-	0.5	1	-	-	-	1.5	2.41	
III	215	-	0.5	1	-	2	-	3.5	±0.35	57.42
	195	-	0.5		1.5	-	-	2		
	185	-	0.5	-	1.5	-	-	2		
	220	-	0.5	1	-	-	-	1.5		
	215	-	0.5	1	-	-	-	1.5		
	185	-	0.5	1	-	-	-	1.5	1.5	
IV	175	-	0.5	-	-	-	-	0.5	±0.22	73.49*

Table: 7. Effect of EECP on Ulcer Index in Acetic acid- Induced Gastric Ulceration

Values are expressed in terms of mean ± SEM of 6 rats (ANOVA)
*Statistically significant $p \leq 0.05$ (in comparison with Standard).



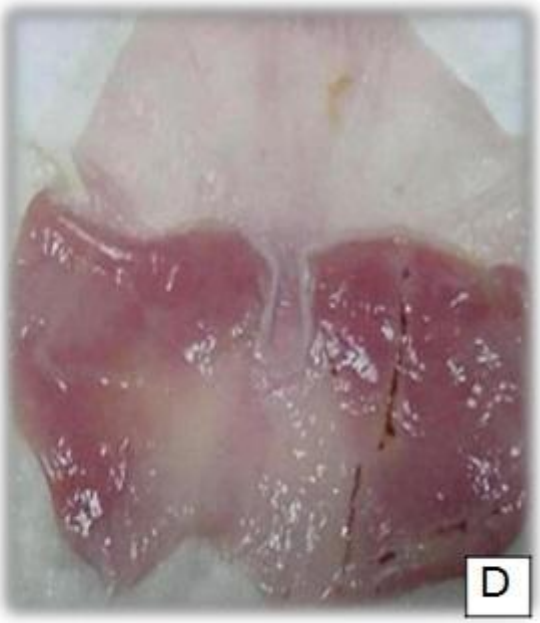
Comparison of all the parameters on Acetic acid induced ulcers

Open excised stomach in Acetic acid induced gastric lesions model:

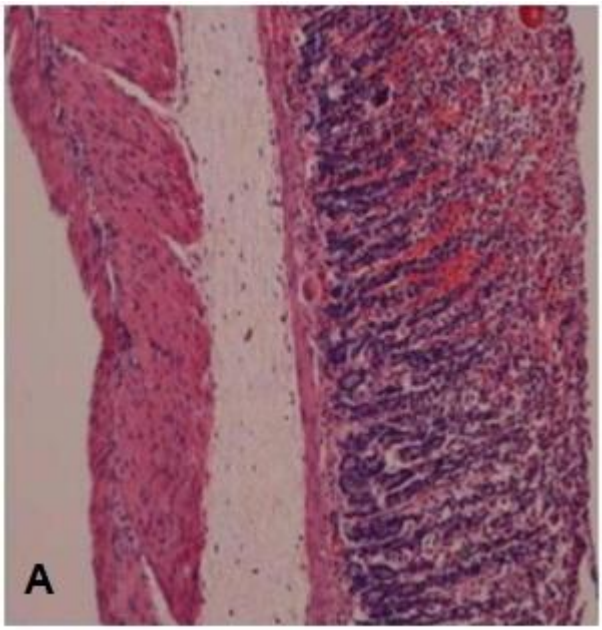
Gastric lesions induced by Acetic acid (2ml

Absence of gastric lesions in Omeprazo (20mg/kg)

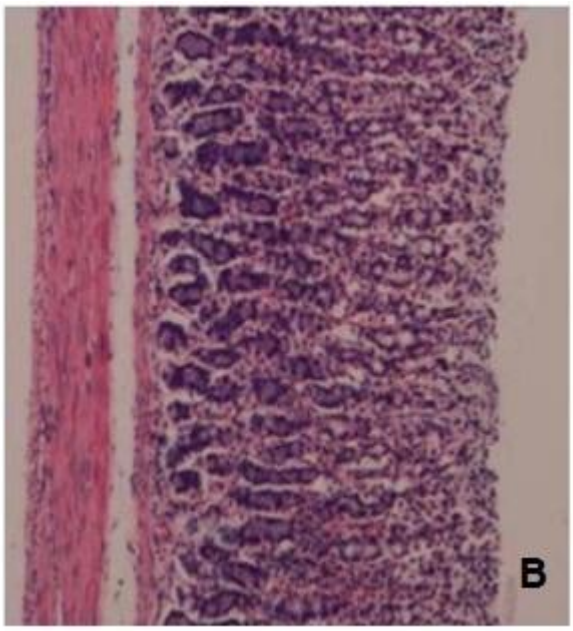
Fraction inhibition in gastric lesions 200mg/kg of EE



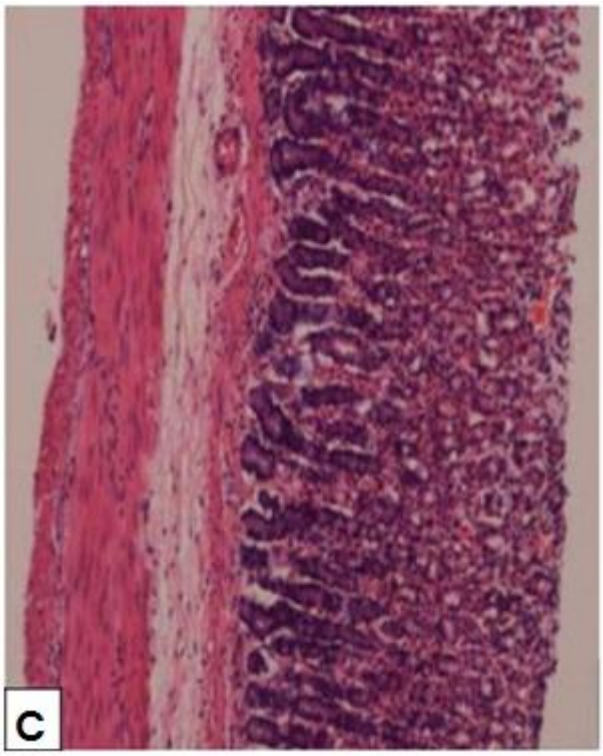
Inhibition in gastric lesions at 400mg/kg EECP



Gastric lesions induced by Acetic acid (2ml



Omeprazole (20 mg/kg) no damage mucosal epithelium was observed.



EE(200mg/kg): Apparent epithelisation wa observed.

EE (400mg/kg): Apparent epithelisatio was observed

Methylene blue Induced ulcerations

Table: 8. Effect of EE on Gastric Volume and pH:

Group N	Body wt. gn	Treatment	Vol. of gastric Juice	pH
I	180	Control	5.0ml	2
	170		5.0ml	2
	175		4.8ml	2
	210		4.4ml	2
	195		4.8ml	2
	190		4.6ml	3
			4.7 ±0.087	2.1 ± 0.152
II	170	Methylene blue 125 mg/kg Omeprazole(20mg/kg)	2.0ml	6
	185		2.2ml	7
	205		1.8ml	7
	185		2.4ml	6
	210		2.8ml	7
	180		2.0ml	5
			2.24 ±0.172	6.3 ±0.304
III	190	Methylene blue 125 mg/kg Ethanolic extract(200mg/kg)	3.0ml	6
	195		3.2ml	6
	180		3.6ml	4
	170		3.4ml	4
	215		3.2ml	6
	205		3.2ml	7
			3.2 ±0.08	5.4 ±0.6*
IV	190	Methylene blue 125 mg/kg Ethanolic extract (400mg/kg)	2.0ml	5
	185		2.8ml	6
	215		2.4ml	6
	190		2.2ml	7
	185		2.8ml	5
	180		3.0ml	6
			2.5 ±0.160*	6 ±0.316**

Values are expressed in terms of mean ± SEM of 6 rats (ANOVA)

*Statistically significant $p \leq 0.05$ (in comparison with Standard).

Table: 9. Effect of EE on Free Acidity and Total Acidity:

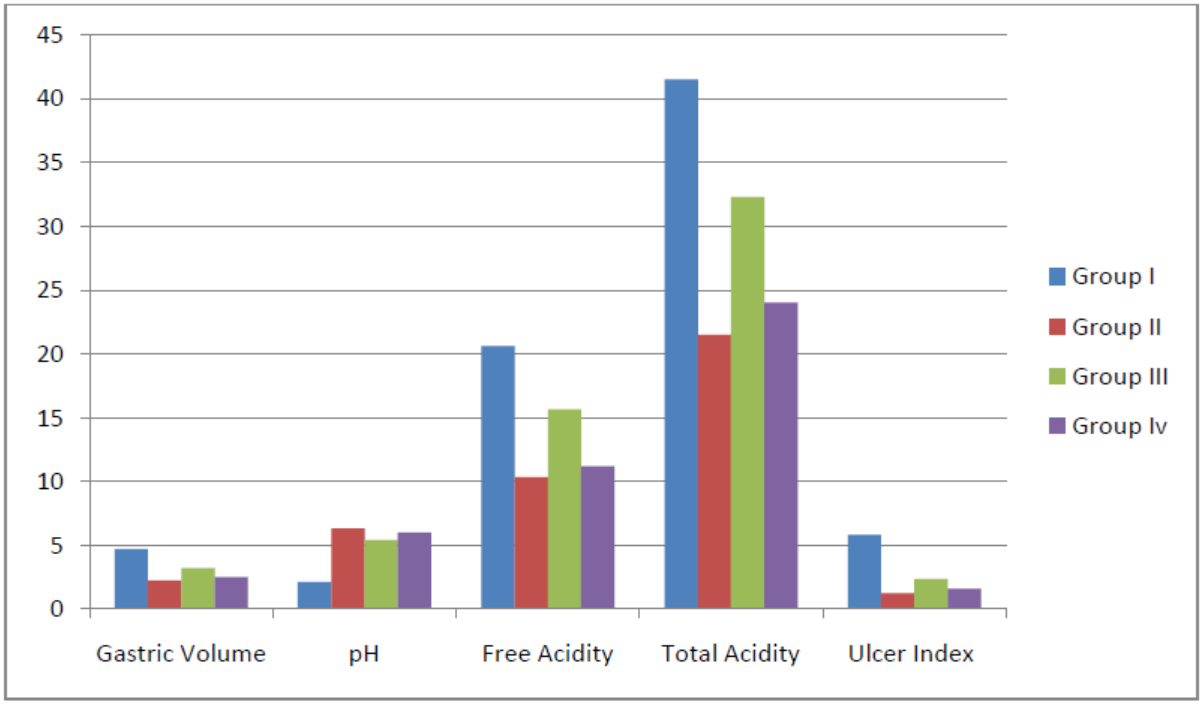
Group No.	Body wt. gms	Treatment	Free Acidity	Total Acidity
I	180		20	42
	170		18	46
	175	Control	21	40
	210		19	42
	195		22	40
	190		24	39
			20.6 ±0.88	41.5 ±.1.02
II	170		10	18
	185		8	26
		Methylene b		
	205	125 mg/kg	12	20
	185	Omeprazole(20mg/kg	10	22
	210		14	19
	180		8	24
			10.33 ±0.95	21.5 ±1.25
III	190		14	32
	195		14	36
		Methylene b		
	180	125 mg/kg	11	34
		Ethanolic		
	170	extract(200mg/kg)	18	32
	215		16	28
	205		18	32
			15.66 ±1.10	32.3 ±1.085
IV	190		12	26
	185		14	24
		Methylene b		
	215	125 mg/kg	12	22
	190	Ethanolic extract	10	22
	185	(400mg/kg)	9	26
	180		10	24
			11.16 ±0.74*	24 ±0.73*

Values are expressed in terms of mean ± SEM of 6 rats (ANOVA)
*Statistically significant P ≤ 0.05 (in comparison with Standard).

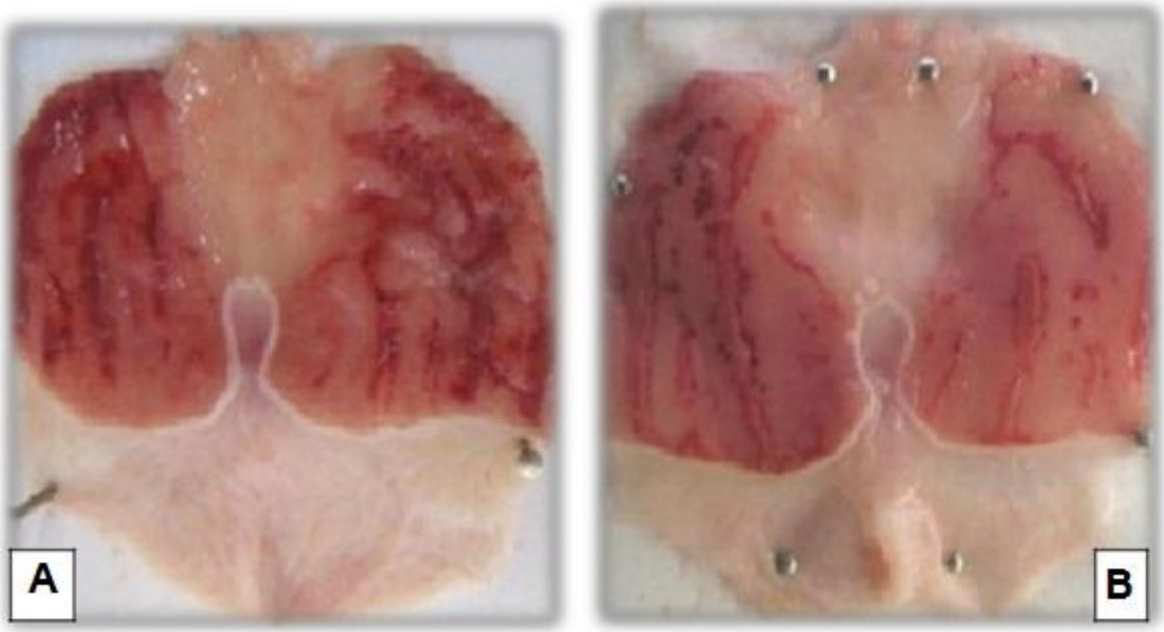
Table: 10. Effect of EECP on Ulcer Index in Methylene Blue -Induced gastric ulceration (Mean ± SE, n=6 in each group)

Group No	Body Weight gm	ULCER INDEX						Total Score	Mean Ulcer Index ± SE	% Protection
		Normal Colour stomach	Normal Colour stomach	Ulcer Spots	Hemorr-agic streaks	Ulcers ≥3 but ≤	Ulcer> 5			
	180	-	0.5	1	1.5	-	3	6		
	170	-	0.5	1	1.5	-	3	6		
	175	-	0.5	1	1.5	-	3	6		
	210	-	0.5	1	1.5	2	-	5	5.83	
	195	-	0.5	1	1.5	-	3	6	±0.1	
I	190	-	0.5	1	1.5	-	3	6	6	-
	170	-	0.5	1	-	-	-	1.5		
	185	-	0.5	-	-	-	-	0.5		
	205	-	0.5	1	-	-	-	1.5		
	180	-	0.5	1	-	-	-	1.5	1.25	
	210	-	0.5	-	1.5	-	-	2	±0.2	
II	180	-	0.5	-	-	-	-	0.5	8	78.55
	190	-	0.5	1	-		-	1.5		
	195	-	0.5	1	1.5	-	-	3		
	185	-	0.5	1	1.5	-	-	3		
	170	-	0.5	1	-	-	-	1.5	2.33	
	215	-	0.5	-	1.5	-	-	2	±0.3	
III	205	-	0.5	1	1.5	-	-	3	7	60.04
	190	-	0.5	-	1.5	-	-	2		
	185	-	0.5	1	-	-	-	1.5		
	215	-	0.5	-	1.5	-	-	2		
	190	-	0.5	-	1.5	-	-	2		
	185	-	0.5	-	-	-	-	0.5	1.58	
IV	180	-	0.5	1	-	-	-	1.5	±0.2	72.89*

Values are expressed in terms of mean ± SEM of 6 rats (ANOVA)
*Statistically significant P ≤ 0.05 (in comparison with Standard).

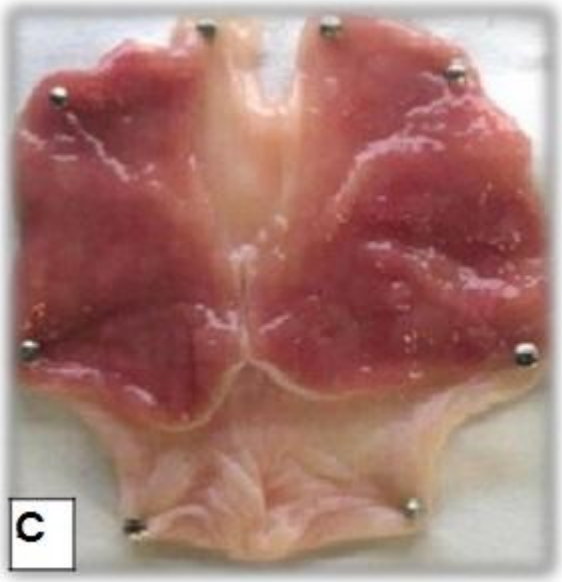


Open excised stomach in methylene blue induced gastric lesions model:

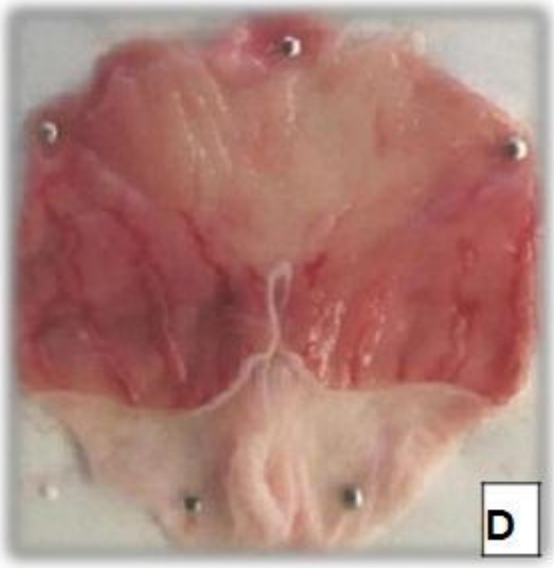


Gastric lesions induced by (30mg/kg)

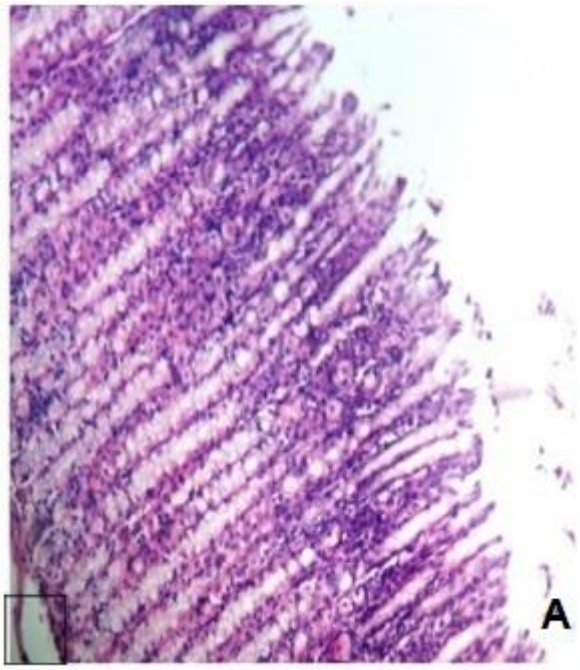
Absence of gastric lesions in Omeprazole (20mg/kg)



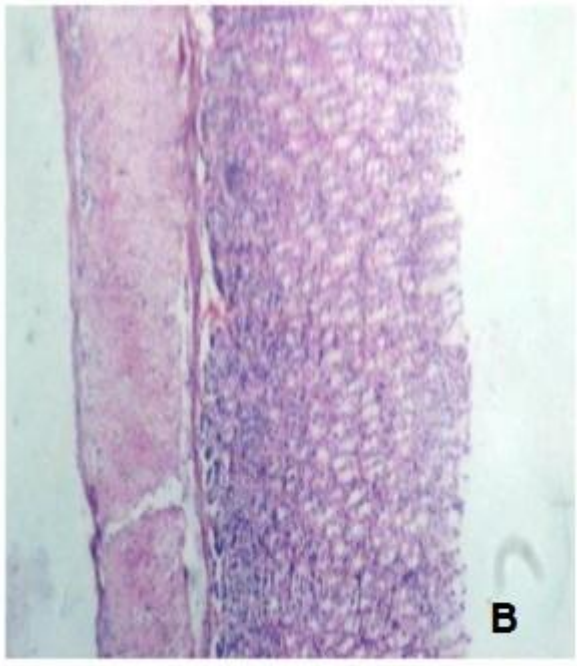
Fraction inhibition in gastric lesions at 200mg/kg of EECP



Inhibition in gastric lesions at 400mg/kg o EECP

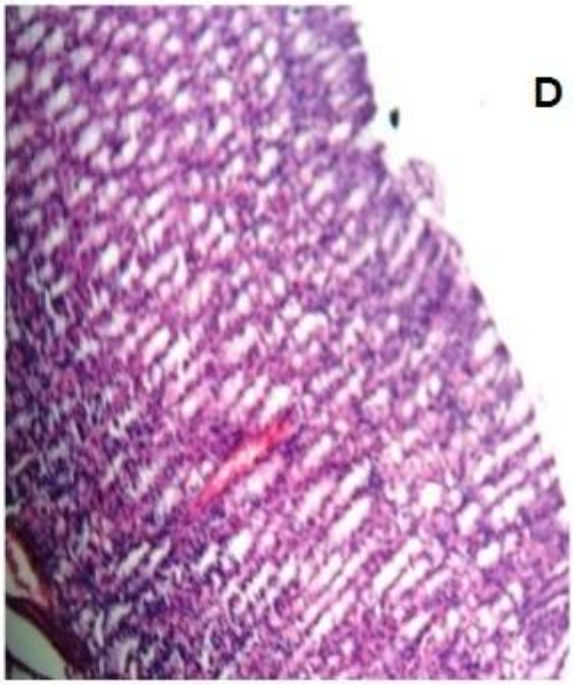


damaged mucosal epithelium wa observed



no damage to mucosal epithelium wa observed.

apparent epithelisation was observed.



apparent epithelisation was observed

ANTI-OXIDANT ACTIVITY

DPPH radicalscavenging assay:
Table: 11. DPPH assay of Ethanolic extract of *Echinops echinatus* Roxb

S. No	Concentration In µg/ml	% of inhibition	IC50 value
1	1000	92.6	47±1.8.*
2	500	78.4	
3	250	71.4	
4	125	62.4	
5	61.5	56.4	
6	31.2	35.9	
7	15.6	15.1	
8	Control	0	
9	Standard Rutin	-	43.60±1.79

Values are the average of (n=3) and represented as mean ± standard deviation and probability value * P ≤ 0.05 significant when IC50 values are compared with standard.

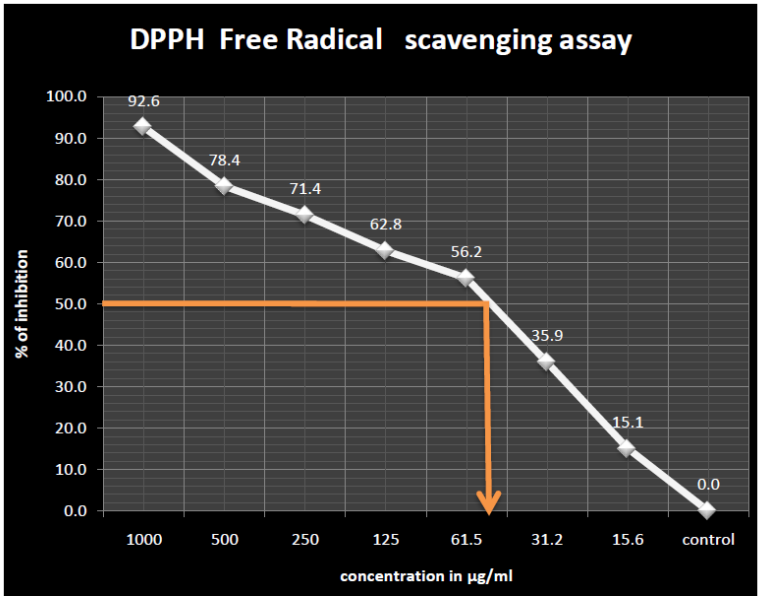


Figure: 25 DPPH radical scavenging assay IC50 value

Nitric oxide scavenging assay:

Table: 12, Nitric oxide radical scavenging activity of Ethanolic extract of *Echinops echinatus* Roxb

S. No	Concentration in µg/ml	% of inhibition	IC50 value
1	1000	68.33	63± 1.6 *
2	500	60.8	
3	250	55	
4	125	54.1	
5	61.5	50.8	
6	31.2	42.5	
7	15.6	27.5	
8	Control	0	
9	Standard Rutin	-	65.44

Values are the average of (n=3) and represented as mean ± standard deviation and probability values

* P ≤ 0.05; significant when IC50 values are compared with standard values.

Nitric oxide scavenging assay:

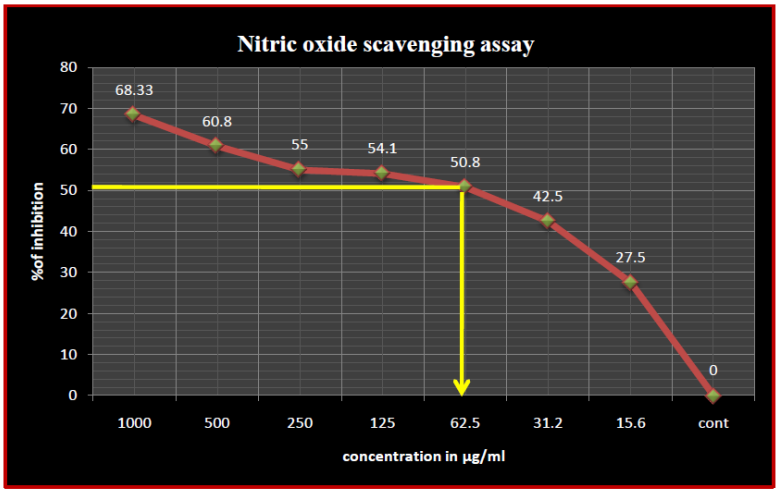


Fig no: 26 Nitric oxide radical scavenging assay IC50 values

VII. DISCUSSION

The etiology of peptic ulcer is unknown in most of the cases, yet it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defence mechanisms. To regain the balance, different therapeutic agents including plant extracts are used (in experimental animals) to inhibit the gastric acid secretion or to boost the mucosal defence mechanisms by increasing mucus production, stabilizing the surface epithelial cells or enhancing prostaglandin synthesis.[72]

Though a very good numbers of anti-ulcers drugs like antisecretory drugs, H2 receptor antagonists, proton pump inhibitors, antimuscaranic, cytoprotectants and Prostaglandins analogues are available; the side effects associated with these drugs limit their use. Many herbal drugs from Ayurveda of Indian traditional system of medicine are advocated for the management of peptic ulcer. Herbal medicines are used as plant extracts from different parts are now a days consider as safe medication for the treatment of a number of diseases as it is a general notion that plant based drugs are safer without any side effects.

Though the presently available anti-ulcer drugs have remarkable effects in ulcer therapy the efficacy is still incomplete as there are many a number of incidences of relapse with adverse effects and drug-drug interaction are reported with the therapy. Hence there is a need for ideal anti-ulcer drugs with extended action from herbal source with a caliber of better protection and low incidence of relapse of ulcers.[78]

In Indian system of medicine a very good numbers of herbs are reported to produce antiulcer and anti-inflammatory activities. Hence in the present study *Echinops echinatus* Roxb (whole plant) posses antiulcer activity in Acetic acid and Methylene Blue induced ulcer model.

The present results demonstrate that the ethanolic extract of *Echinops echinatus* Roxb whole plant protect the rat gastric mucosa against hemorrhagic lesion produced by acetic acid and methylene blue. These inducing methods of gastric lesions are rapid and convenient way of screening plant extracts for antiulcer potency and cytoprotection in macroscopically and microscopically visible lesions.[89]

acetic acid and methylene blue induced gastric ulcers has been widely used for the experimental evaluation of antiulcer activity. Acetic acid induced and Methylene Blue induced gastric lesion formation may be due to stasis in gastric blood flow, which contributes to the development of the hemorrhagic and necrotic aspect of tissue injury. NSAID's like acetic acid causes gastric mucosal damage by decreasing prostaglandin levels through inhibition of PG synthesis.[92]

Tannins83, flavonoids84, glycosides85, triterpenes86 and Phytosterols87 are reported for their anti-ulcer activity. All the above mentioned phytoconstituents are present in *Echinops echinatus* Roxb and hence might have contributed for anti ulcer activity.[94]

Echinops echinatus Roxb has significantly protected the gastric mucosa against acetic acid and methylene blue challenge as shown by reduced values of lesion index as compared to solvent control group suggesting its potent cytoprotective effect. It shows significant inhibition of formation of gastric ulcer and also decreases both acid concentration, gastric volume and increased the pH values. It suggests that Ethanolic extract of *Echinops echinatus* Roxb can suppress the gastric damage induced by aggressive factors.

Echinops echinatus Roxb extract was significantly effective in protecting gastric mucosa against Acetic acid induced ulcers at different dose level studied. Methylene Blue induced gastric injury is associated with significant production of oxygen free radicals leading to increased lipid peroxidation, this causes damage to cell and cell membrane.[96]

Reactive oxygen species, reactive nitrogen species, reactive carbonyl species are products of normal cellular metabolism, act as a secondary messenger controlling various normal physiological functions of organs. Oxidative stress seems to be cause many diseases particularly in inflammation, diabetes, cancer, gastric disorders and other many other diseases. This situation motivates research on naturally occurring anti-oxidants from plants, organism known to have developed efficient systems that protect them against environmental oxidative stress. Bio molecules of remarkable structural diversity, plant anti-oxidants are results of millions of years of evolutionary optimization, with view to achieving a perfect functionality.

The free radical scavenging activity of extract of Echinops echinatus Roxb. was evaluated based on the ability of quench the synthetic DPPH. The assay provides useful information on reactivity of the compounds with free radicals. DPPH absorption represents the capacity of test compound to scavenge free radical independently. The extract possessed significant DPPH radical scavenging activity compared with standard rutin.

Nitric oxide is a mediator of physiological processes such as smooth muscle relaxation, neuronal signalling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is diffusible free radical which plays many roles as effectors molecules in diverse biological systems including vasodilatation, antimicrobial and antitumor activity. Scavenging of nitric oxide compete with oxygen leading to the reduced production nitric oxide.The scavenging activity of *Echinops echinatus* Roxb extract shows significant effect on anti-oxidant activity. The extract of Nitric oxide scavenging activity compared with standard rutin.[89]

The phytochemical screening of the extract of *Echinops echinatus* Roxb revealed the presence of alkaloids, flavonoids and Triterpenoids. Any of these metabolites may be responsible for the anti-oxidant and anti-ulcer properties of *Echinops echinatus* Roxb

VIII. CONCLUSION

From the present study it was concluded that the preliminary phytochemical evaluation of Ethanolic extract of *Echinops echinatus* Roxb revealed the presence of tannins, sterols, flavonoids, glycosides, alkaloids and triterpenes.

Acute oral toxicity studies shows no mortality recorded with the extracts even at the dose level of 2000 mg/kg body weight.

From the results of present study it was concluded that the ethanolic extract of *Echinops echinatus* Roxb (whole plant) at 400mg/kg shows significant anti-ulcer activity in animals models used, as compared to the standard drug Omeprazole. The positive anti-oxidant activities in different methods further confirm its protection against gastro duodenal pathogenesis.

The present study clearly demonstrated the antiulcer and antioxidant property of ethanolic extract of *Echinops echinatus* Roxb. These effects may be due to the presence of tannins, alkaloids, flavonoids, and triterpenes observed in the extract.

These finding thus prompt further necessary study to carry out the elucidation of mechanism of actions related to anti ulcer and anti oxidant activity in molecular levels, by which more effective treatment for the disorders can be achieved.

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APPROVAL CERTIFICATE

This is to certify that the project title "Evaluation of toxicity and antiulcer activity of ethanolic extract of aerial parts of *Echinops Echinatus* Roxb" has been approved by IAEC and the details are furnished under

Approval no	Name of the species	Breakup sex wise	Weight	No's approved
SVCE/BIO/2018/011	Wistar Albino rat.	23 female& 20 male	150-200gms	43
Forty three wistar albino rats only				

31/5/18

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AUTHENTICATION CERTIFICATE

Based upon the organoleptic / macroscopic / microscopic examination of Sample, it is certified that the specimen given by **SYEDIKRAMSHARIF SR of Mohamed Sathak A.J College of Pharmacy** is identified as below:

Plant Name	: *Echinops echinatus*
Family	: *Asteraceae*
Kingdom:	: *Plantae*
Subkingdom:	: *Tracheobionta*
Division:	: *Magnoliophyta*
Class:	: *Magnoliopsida*
Subclass:	: *Asteridae*
Order:	: *Asterales*
Family:	: *Asteraceae*
Genus:	: *Echinops*
Species:	: *echinatus*

Reg.No of the certificate : PARC/1713-2018-19

References : Nair, N.C & Henry, A.N. Flora of Tamilnadu, India 1 : .1983.
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Theme: Innovations in Pharmaceutical and Biological Research: Discovering Novel Approaches

February 23-24, 2018

Venue: Convention Center, B.S. Abdur Rahman Crescent Institute of Science & Technology, Chennai [Tamil Nadu] India

Certificate

This is to certify that Prof./Dr./Mr./Mrs./Ms. **Syed Ikram Sharif S R**

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